

ARO 49th MidWinter Meeting

PUERTO RICO CONVENTION CENTER
FEBRUARY 7-11, 2026

Abstract Book



**SAN JUAN,
PUERTO RICO**

Friday, February 6, 2026

gEAR Session: Beginner Workshop - Introduction to gEAR

7:00 p.m. - 9:00 p.m.

Meeting Room 208ABC, Floor 2

Presenters:

Joshua Orvis, *Institute for Genome Sciences*

Daniel Lesperance, *Institute for Genome Sciences, University of Maryland, Baltimore*

Session Overview: This introductory workshop provides a hands-on tour of the Gene Expression Analysis Resource (gEAR). Participants will learn how to create an account, navigate the user dashboard, and search across gEAR's growing library of datasets. We'll cover the basics of finding and viewing gene expression profiles, organizing results into lists, and exploring curated dataset collections.

Shifting then to more advanced applications, we will focus on tools like the single-cell workbench, spatial displays, and epigenomic browser and projection, where users can dive deeper into their multi-omic data. You'll learn how to visualize clusters, compare cell populations, and quickly identify expression patterns across tissues and developmental stages. By the end of the session, attendees will have the tools to confidently begin using gEAR to support their own research questions.

This workshop includes a gEAR binder with all the instructional materials, and snacks and refreshments for the long session.

Saturday, February 7, 2026

Presidential Symposium: The Power of Collaboration - Part 1

Chair: Ronna Hertzano, *National Institutes of Health*

8:00 a.m. - 9:30 a.m.

Ballroom B, Floor 3

Session Overview: This year's Presidential Symposium celebrates the transformative impact of collaboration, how breaking boundaries, sharing expertise, and building trust create a synergy in which the whole becomes greater than the sum of its parts. Inspired by breakthroughs in single-cell analysis and accelerated progress in clinical trials across our field, the symposium, *The Power of Collaboration*, is organized into two thematic parts. Each segment opens with an inspiring plenary lecture, followed by a panel of concise, high-impact presentations that exemplify collaborative science in action.

A Community Standard Cell Type Atlas of the Brain: The Brain Initiative Cell Atlas Network

Mike Hawrylycz*¹

¹*Allen Institute for Brain Science*

Individual Abstract: Single-cell genomics has revolutionized our ability to construct comprehensive cellular atlases of the brain, enabling researchers to systematically characterize the molecular diversity of individual neurons and glia at unprecedented resolution. Cell type atlases and databases allow the creation of molecular reference standards akin to the pangenome, a standardized representative sequence derived from the genomes of multiple individuals that act as a genetic baseline for comparison. This paradigm underpins the work of the BRAIN Initiative Cell Census Network (BICAN), which is creating a standardized and integrated cell atlas of the human, macaque, marmoset and mouse brain as a new community standard to serve as a unifying reference for the mammalian brain. Brain atlases have now been generated for diverse species, regions, and disease states and form invaluable resources for studying cell types and their perturbations. Through this work and active consortia such as BICAN, the Human BioMolecular Atlas Program (HuBMAP) and the Human Cell Atlas (HCA) we are rapidly proceeding toward a comprehensive reference map of all human cell types—including their molecular profiles, spatial locations, developmental trajectories and inter-cellular interactions. The brain and the ear are closely connected through the auditory and vestibular systems and with connections to the primary and peripheral nervous system the ear has essential links to healthy brain function. A single-cell ear atlas would transform hearing and balance research by defining every molecular cell type, linking genotype to function and pathology, and providing a reference framework for regenerative and therapeutic innovation, much as brain and retinal atlases have done for their respective systems. In this presentation we survey the techniques and accomplishments of cell type characterization in the brain and peripheral nervous system highlighting opportunities for a constructing detailed map of the auditory and vestibular systems.

Gearing up for Regeneration

Lisa Goodrich*¹, John V. Brigande², Alain Dabdoub³, Albert Edge⁴, Andrew K. Groves⁵, Ksenia Gnedeva⁶, Stefan Heller⁷, Ronna Hertzano⁸, Tatjana Piotrowski⁹, David W. Raible¹⁰, Yehoash Raphael¹¹, Litao Tao¹², Mark Warchol⁵, Seth A. Ament¹³

¹*Harvard Medical School*, ²*Oregon Hearing Research Center, Oregon Health and Science University*, ³*Sunnybrook Research Institute, University of Toronto*, ⁴*Massachusetts Eye and Ear Infirmary, Harvard Medical School*, ⁵*Washington University School of Medicine*, ⁶*University of Southern California*, ⁷*Stanford University, Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine*, ⁸*Institute for Genome Sciences, University of Maryland School of Medicine*, *National Institute on Deafness and Other Communication Disorders, Intramural Research Program*, ⁹*Stowers Institute for Medical Research*, ¹⁰*University of Washington*, ¹¹*University of Michigan*, ¹²*Creighton University*, ¹³*Institute for Genome Sciences, University of Maryland School of Medicine, Maryland Psychiatric Research Center, University of Maryland School of Medicine*

Individual Abstract: With the introduction of out-of-the-box methods to generate high-quality genomic data even from rare cell populations, inner ear researchers now have access

to many large datasets that can be used to answer a wide range of experimental questions and generate hypotheses. However, it can be challenging to access or analyze data effectively, especially when methods and quality vary across labs. The Hearing Restoration Project (HRP) was founded on the core principle that collaboration will accelerate and improve the field's efforts to find molecular entry points for stimulating hair cell regeneration. To lower the barrier for everyone, HRP has produced a large number of single cell and single nucleus datasets from all hair cell bearing organs from four species across development and after damage. 13 laboratories are currently working together to develop and apply cutting edge bioinformatic approaches that can be used to identify key nodes of gene expression and mechanisms of regulation, to develop standardized methods for screening candidate pathways in vivo and in organoids, and to test directly how manipulation of candidate pathways can improve the number and maturity of hair cells regenerated from supporting cells. Efforts to date have revealed cohorts of genes that are enriched in hair cells relative to supporting cells across species or that are associated with key steps in regeneration. Data generated by HRP researchers is freely available through gEAR, where you can directly query the results of our analysis to further your own research goals.

Towards a High-Resolution Spatiotemporal Transcriptomic Atlas of the Vestibular Utricle

Rachel Honeyghan-Williams^{*1}, Jimena Perez Lloret², Valeria Castagna¹, Marcela Lipovsek¹

¹*University College London*, ²*University College London Ear Institute*

Individual Abstract: The cellular composition of vestibular sensory epithelia has been extensively explored using histological and electrophysiological methods, identifying two major types of hair cells, type I and type II, and two distinct areas on the epithelium, the striola and extrastriola. Single cell transcriptomics has been able to match overall gene expression signals to this wider cell type and spatial definitions. However, due to the challenges inherent to the preparation of single cell suspensions, the transcriptomic profiling has been, with few exceptions, low-throughput and focused on early postnatal stages.

Here, we present a novel tissue collection and freezing strategy that, coupled with the preparation of single nuclei suspensions and combinatorial barcoding sequencing libraries, allowed us to generate, in an experimentally flexible and cost-effective way, a high-resolution dataset that profiles the mouse utricle from embryonic to adult stages, encompassing 17 timepoints and ~120,000 cells.

By implementing non-linear dimensionality reduction and visualisation tools, cluster stability evaluation and non-negative matrix factorisation for gene module analysis we have reached robust and granular transcriptomic definitions of the cell types and subtypes that make up the vestibular utricle. Moreover, trajectory and RNA velocity analysis are allowing us to study in detail the maturational process of individual cell types and their subcellular functional modules.

We will discuss how this in-depth exploration of the cell types and subtypes present in the mouse utricle can be exploited to further our understanding of the functional maturation and potential for sensory hair cell regeneration in inner ear sensory epithelia.

HIEDRA: A Human Inner Ear Development Single-Nucleus RNA Atlas

Heiko Locher^{*1}

Individual Abstract: The human inner ear comprises a diverse array of highly specialized cell types that enable hearing and balance. Recent studies have advanced our understanding of their development, yet human-specific mechanisms are still being uncovered. This gap limits our ability to interpret how genetic variants cause inner ear disorders and to develop targeted therapies.

To address this, we generated HIEDRA (Human Inner Ear Development RNAseq Atlas), a high-resolution single-nucleus RNA-sequencing atlas of the developing human inner ear spanning fetal weeks 7 to 15, capturing both auditory and vestibular domains. HIEDRA identifies many transcriptionally distinct cell populations, including sensory and non-sensory epithelia, neuronal, and mesenchymal cells. Among these are previously under-characterized secretory cells such as the marginal cells of the stria vascularis and the vestibular dark cells, crucial for endolymph homeostasis and inner ear function.

HIEDRA was validated using newly generated data from manually separated cochlear and vestibular tissues (fetal week 12), publicly available mouse cochlear and ampullary datasets (E16), and pseudotime analyses revealing temporal expression of established gene markers. By integrating disease gene annotations, we mapped known deafness and balance disorder genes to specific cell types, revealing selective cellular vulnerabilities of sensory and secretory cell types. We further defined developmental trajectories and gene regulatory networks underlying sensory and secretory lineage specification.

To demonstrate HIEDRA's utility as a discovery platform, we applied its insights to human inner ear organoids, aiming to induce the formation of secretory cells, cell types normally absent in this model. We found that suppression of Hedgehog signaling is essential for secretory cell fate acquisition. Targeted Hedgehog inhibition successfully promoted formation of vestibular dark cells and cochlear marginal cells, enhancing organoid complexity and establishing a more physiologically relevant in vitro model for studying disease mechanisms and testing therapeutic interventions.

Altogether, HIEDRA offers a human-specific framework for understanding inner ear development and disease, serving as an open resource for the research community.

Comap – A Single Cell “Omic” Atlas of the Cochlea Across the Life Span

Matthew Kelley*¹, Tessa R. Sanders¹, Elizabeth C. Driver¹, Beatrice Milon¹, Christopher Shults¹, Amanda Bonczkowski¹, Rafal Olszewski¹, Wei Song¹, Nachiket Kelkar¹, Katherine Fernandez¹, Lisa Cunningham¹, Ran Elkon¹, Michael Hoa¹, Robert Morell¹, Ronna Hertzano¹

¹*NIH/NIDCD*

Individual Abstract: Background: The cochlear duct is a remarkable structure. While small in size (30 mm long in human, 6 mm long in mice), the cochlea successfully detects and processes sounds across a wide range of frequencies and sound intensities. The cochlea's exceptional performance is achieved, in part, through its highly diverse population of cell types, which are distributed in stereotypical patterns within the duct. Despite the importance of these varied cell types for auditory function, the small size of the cochlea and its high cellular diversity have prevented a full characterization of their gene expression and epigenetic states.

Methods: To address this challenge, we will combine spatial transcriptomics with single cell RNA and ATAC sequencing at different time points to create an omic atlas of the developing, adult, and aging cochlea. The resulting data will be posted to the gEAR website in a user-friendly format that will allow researchers to easily determine gene expression and epigenetic state for cells located in specific locations within the cochlea.

Results: Using the CBA/CaJ mouse line, which does not experience premature age-related hearing loss, we are collecting data from 5 embryonic and early post-natal time points prior to the onset of hearing. In addition, we will collect cochleae at P14, P30 and > P365 to characterize omic profiles during normal function and aging. As a first step, we have generated and posted on gEAR the data for cochleae at post-natal day 1.

Conclusions: Cellular diversity, function, and degeneration are all, ultimately, driven by the expression or lack of expression of specific genes or gene isoforms. By creating an in-depth single cell atlas of cochlear gene expression and epigenetic states throughout the mouse lifespan, we plan to provide a baseline dataset that can be used to characterize genetic changes underlying both known and unknown causes of auditory dysfunction.

NIDCD's Human Temporal Bone Resources

Amy Poremba*¹, Janet Cyr¹, Troy Hackett¹

¹*NIH-NIDCD*

Individual Abstract: As the delicate inner ear is encased within the temporal bone, one of the hardest bones of the body, detailed study of the human inner ear relies on post-mortem temporal bones to understand underlying pathophysiology which is critical for illumination of how humans process hearing and balance information. At NIDCD (National Institute on Deafness and Other Communication Disorders), we have formed a three-part resource for studying the human temporal bone/inner ear tissue: 1) The National Hearing and Balance Organ Donor Program to coordinate a list of potential consented donors, and then procure and distribute the inner ear tissues to be processed for researchers. This resource is managed by the Human Tissues and Organs for Research Resource through the National Disease Research Interchange; 2) The National Human Ear Resource Network for tissue processing, case analysis, method optimization, and data and specimen sharing. There are five currently NIDCD funded laboratories across the nation that comprise this collaborative Network and human inner ear resource. These laboratories are located at Johns Hopkins University, Mass Eye and Ear, Stanford University, University of California, Los Angeles and the University of Minnesota; and 3) EarBase, a searchable database of temporal bone tissue with medical records and archived digital images, which is developed and coordinated by a resource awarded to the University of Southern California, cofunded with the National Institute on Dental and Craniofacial Research and the NIH Office of Data Science Strategy as part of the collaborative FaceBase endeavor. NIDCD is dedicated to encouraging the scientific community to continue to build and maintain national human temporal bone resources for scientific researchers and clinicians and this presentation will describe the three-part synergistic resource and the current status of each part. These overall resources are critical to advance human auditory and vestibular research to understand the underlying pathophysiology of disorders and the discovery of treatments.

Presidential Symposium: The Power of Collaboration - Part 2

Chair: Ronna Hertzano, *National Institutes of Health*

10:00 a.m. - 12:00 p.m.

Ballroom B, Floor 3

Session Overview: This year's Presidential Symposium celebrates the transformative impact of collaboration, how breaking boundaries, sharing expertise, and building trust create a synergy in which the whole becomes greater than the sum of its parts. Inspired by breakthroughs in single-cell analysis and accelerated progress in clinical trials across our field, the symposium, *The Power of Collaboration*, is organized into two thematic parts. Each segment opens with an inspiring plenary lecture, followed by a panel of concise, high-impact presentations that exemplify collaborative science in action.

Preparing for Precision Medicine: The PD GENERation Experience

James Beck*¹

¹*Parkinson's Foundation*

Individual Abstract: Parkinson's disease (PD) is a progressive neurologic disease affecting nearly 1 million in people in the US alone and is without a disease modifying therapy. The cause of PD is unknown for the majority of those affected, but in 1997 the first genetic forms of PD were identified and now dozens of genes are linked directly or indirectly to the disease. Though many of these genes are exceedingly rare, 10-15% of PD patients carry variants in one of seven well-known PD genes. Clinical trials targeting genetic-PD have been repeatedly attempted in the past decade but have failed because of considerable challenges in recruitment. Appreciably complicating the issue is that neither PD patients nor their clinicians know their genetic status. Physician surveys demonstrated that genetic testing was rarely offered to PD patients due to issues around insurance reimbursement, clinical utility, and a perceived lack of patient interest. Nevertheless, clinical development of gene-targeted therapies rapidly continues with nearly a dozen about to enter or in early phase clinical trials for PD.

To overcome this challenge and to accelerate precision trials for PD, the Parkinson's Foundation created the PD GENERation study (NCT04994015), which offers no-cost genetic testing and counseling to almost all diagnosed with PD. In parallel to patient outreach, the Foundation also offered physician education around genetic testing and return of results to bolster clinician knowledge and involvement. PD GENERation started in late 2019 with 6 US sites and now has more than 70 sites in 9 countries across the Americas and in Israel plus the study offers remote enrollment. Patient interest has been robust from the outset with waiting lists forming at one point. Enrollment averages 917 per month and nearly 30,000 participants have been recruited. Recent physician surveys have shown an increased comfort in genetic testing and counseling and a concomitant increase in patient referral. The experience of the PD GENERation study represents a path forward for other disease areas where precision therapies are being developed yet genetic testing is not standard of care.

PD GENERation is sponsored by the Parkinson's Foundation with support from the Global Parkinson's Genetics Program (GP2), a program of the Aligning Science Across Parkinson's (ASAP) initiative.

Lessons Learned From Genetic Testing for Hearing Loss in the USA

Richard Smith¹, Amanda Odell², Kevin T Booth³, Diana Kolbe², Thomas Casavant⁴, Brian Hong⁴, Michael J. Schnieders⁴, Terry Braun⁴, Hela Azaiez⁴, Hela Azaiez*⁵

¹*The University of Iowa Hospitals and Clinics*, ²*The University of Iowa Hospitals and Clinics, Molecular Otolaryngology and Renal Laboratories*, ³*Harvard Medical School*, ⁴*The University of Iowa*, ⁵*Indiana University*

Individual Abstract: Genetic testing has become an essential diagnostic tool in the USA in the evaluation of deaf and hard-of-hearing individuals. It delivers actionable information for prognosis, family counseling, and management, but it also exposes persistent technical and interpretive challenges. Disease-specific variant curation and multidisciplinary review substantially enhance diagnostic yield by prioritizing clinically relevant variants and recovering pathogenic or likely pathogenic variants that automated pipelines may filter out.

Systematic aggregation of deafness-associated variation through a large-scale genetic testing program involving more than 8000 individuals and resources like the Deafness Variation Database (DVD) reveal striking heterogeneity in mutational architectures across genes and protein domains rather than a uniform genome-wide pattern of constraint. Notably, genes such as GJB2 and SLC26A4 demonstrate high coding-region variant rates accompanied by a disproportionate fraction of pathogenic variants, a pattern that challenges the reliability of generic population constraint metrics and highlights the need for disease- and gene-specific variant interpretation frameworks.

Many hearing-related proteins have structurally and functionally distinct regions, and as such, domain-level analysis provides critical refinement for variant interpretation. Pathogenicity often depends on variant position, transcript context, and whether truncating alleles escape nonsense-mediated decay. Notable examples including DIAPH1 and COL11A1 illustrate how identical variant classes may produce distinct phenotypes or modes of inheritance depending on their location and functional impact. Furthermore, variant-type distribution differs substantially by gene. These gene-specific mutational signatures complicate automated pathogenicity prediction, inflate the proportion of variants of uncertain significance (VUS), and demand improved computational tools and targeted functional assays for accurate reclassification.

Integrating large-scale genomic data from patient cohorts into variant curation frameworks improves diagnostic accuracy, reduces VUSs, and refines genotype–phenotype correlations. Operational practices to improve outcomes include incorporation of deafness-specific databases like the DVD into annotation pipelines, routine multidisciplinary genomic case review to integrate phenotype with genotype, and retention of low-quality or ambiguously mapped variants for expert reassessment rather than automatic exclusion. Such practices reduce false negatives, clarify gene-phenotype relationships, and guide appropriate follow-up testing or referral for gene-targeted therapies.

Persistent gaps remain: the noncoding and regulatory landscape of deafness is under characterized, functional validation of many recurrent missense VUSs is lacking, and national harmonization of reporting standards and data-sharing infrastructures is limited. Addressing these gaps will require continued investment in curated databases, coordinated efforts to fund functional studies, standardized clinical reporting, expanded disease-based reference resources, and the development of domain- and transcript-aware pathogenicity models. Coupled with clinician education, accessible genetic counseling, and integration into newborn

hearing screening pathways, these strategies will greatly improve outcomes for individuals with hearing loss.

Acknowledgement: This work was supported in part by National Institutes of Health—National Institute on Deafness and Other Communication Disorders grants R01 DC002842, DC003544, and DC012049.

Genetic Architecture of Deafness in Mainland China Revealed From a Nationwide Genetic Cohort Study Involving 22,125 Cases

Huijun Yuan^{*1}, Jing Cheng², Yu Lu², Mingjun Zhong², Jia Geng², Fandi Ai², Gen Zhang², Fengxiao Bu²

¹*West China Hospital*, ²*Institute of Rare Diseases, West China Hospital of Sichuan University*

Individual Abstract: Over the past decade, the Chinese Deafness Genetics Consortium (CDGC) has conducted a nationwide genetic cohort study encompassing 22,125 individuals with hearing loss. In this cross-sectional study, participants were recruited from 91 special-education schools, 89 rehabilitation centers for deaf children, and 34 hospitals, covering 94.3 % of prefecture-level regions and 41 ethnic groups across mainland China. A three-stage genetic-testing strategy was implemented and enhanced by the Framework of Iterative Analysis (FIA) and the Recessive Genotype Network (RGnet) to improve variant interpretation. This nationwide effort linked detailed clinical phenotypes with comprehensive genomic data, enabling population-scale dissection of the genetic architecture of hereditary deafness.

Among the 22,125 individuals with complete analysis, most were affected by early-onset (96.0 %), severe/profound (94.7 %), and nonsyndromic (98.8 %) deafness. A molecular diagnosis was achieved in 50.1 %, implicating 110 deafness-associated genes. Notably, 96.6 % of diagnoses were attributable to 61 of 79 genes identified before 2005, whereas 49 of 122 genes discovered between 2005 and 2021 accounted for only 3.4 %. Among early-onset severe/profound cases (n = 19,062), the diagnostic yield reached 50.6 %, compared with 30.6 % in late-onset cases. GJB2 was the most frequently implicated gene (40.7 %) and, together with SLC26A4, accounted for 69.3 % of all diagnoses. The top ten genes (GJB2, SLC26A4, MYO15A, MT-RNR1, MYO7A, MITF, CDH23, TMC1, SOX10, and OTOF) represented 88.4 % of all genetic diagnoses, with marked regional variation (e.g., SLC26A4 in Guangxi, MYO15A in Fujian, MT-RNR1 in Qinghai).

Within the cohort, 111 individuals carried biallelic pathogenic or likely pathogenic variants in OTOF, corresponding to a 0.54 % positivity rate in the total cohort and 1.11 % among genetically diagnosed cases. The most recurrent variants were NM_001287489.1:c.5975A > G, c.2977_2978del, and c.2215-1G > C. The overall genetic contribution to early-onset severe/profound deafness was estimated at 67.7 %. Outcome data from cochlear-implant recipients linked to 80 deafness genes further informed genotype-based surgical counseling.

To refine interpretive accuracy, the RGnet and FIA frameworks together improved diagnostic yield by 7.6 % through cohort-informed variant interpretation. A distinct latitudinal gradient and a high population burden of GJB2 p.V37I were observed, showing quantitative association with severe/profound hearing loss. The prevalence of mitochondrial MT-RNR1 variants (m.1555A > G and m.1494C > T) declined markedly across successive birth cohorts, in parallel with national aminoglycoside-stewardship policies.

Collectively, this nationwide, data-driven effort delineates the comprehensive genetic landscape of deafness in China, establishes scalable analytic frameworks for variant interpretation, and illustrates how large-scale collaboration can transform Mendelian-disease research into a continuously learning healthcare system.

Advancing Genetic Testing for Hearing Loss in Puerto Rico: Addressing Systemic Barriers and Building a Path Toward Equitable Genomic-Based Care

Elinette Albino¹

¹Universidad De Puerto Rico

Individual Abstract: Genetic testing has transformed the diagnostic landscape for childhood hearing loss, enabling earlier identification of etiologies, more precise clinical management, and improved genetic counseling. However, despite the existence of a robust Newborn Hearing Screening Program, access to genetic testing in Puerto Rico remains limited, and the integration of genomic tools into routine clinical care is inconsistent. This presentation will describe the current state of genetic testing for hearing loss on the island, drawing on data from ongoing research initiatives and community partnerships, and will highlight the systemic barriers that hinder equitable access to testing and clinical genetics services.

Puerto Rico faces several entrenched obstacles that restrict the implementation of evidence-based genetic evaluations. These include scarcity of on-island clinical genetics providers, long wait times for evaluations (often exceeding 6–12 months), fragmented referral pathways, limited insurance coverage for molecular testing, and non-existing local clinical laboratory capacity for next-generation sequencing. Geographic disparities, socioeconomic constraints, and the lack of a centralized registry further contribute to unequal access across regions. Families frequently report diagnostic odysseys that span years, leading to delayed interventions and missed opportunities for targeted care. These challenges disproportionately affect Deaf and Hard of Hearing children from underserved communities, amplifying health inequities in a population already underrepresented in genomic research.

Our ongoing project, Genetics and Audiological Profiles of Hearing Loss in Puerto Rico, aims to bridge these gaps by integrating exome and genome sequencing with comprehensive audiological and vestibular assessments. Through partnerships with NIDCD, local audiology clinics, and community organizations, we are developing a population-specific genomic reference framework for Puerto Rican individuals. Preliminary findings highlight the presence of potentially novel or rare variants not well represented in existing databases, underscoring the importance of studying genetically admixed populations such as Puerto Ricans. Community engagement, particularly with Deaf and Hard of Hearing families, has also revealed pressing needs related to genetic literacy, culturally appropriate counseling, and clarity about the implications of genomic results.

Together, these efforts demonstrate the urgent need for systemic improvements in clinical genetics infrastructure, insurance coverage, provider training, and patient-centered communication strategies. Expanding access to molecular diagnostics in Puerto Rico will require multisector collaboration and sustained investment, but the potential impact on early detection, family planning, and targeted therapeutics is profound.

This presentation will situate Puerto Rico within global genomic initiatives and illustrate how population-specific data, community partnerships, and equitable implementation strategies can advance hearing-loss genomics in historically underserved populations.

Introducing HearGene Connect, a Nationwide Registry and Natural History Protocol to Correlate Genetic Variants With Phenotypic Outcomes and Therapeutic Eligibility

Isabelle Roux^{*1}, C. Brewer¹, K. Abbey¹, G.L. Poling¹, H. Azaiez¹, J. Sapp¹, R. Faridi¹, S. Haines¹, M. Hoa¹, W. Chien¹, N.M. Biassou¹, O. Shchelochkov¹, W. Zein¹, T.B. Friedman¹, A. Mahurkar¹, R. Hertzano¹

¹*NIDCD/NIH*

Individual Abstract: Genetic testing for hearing loss offers clear clinical benefits, including early identification of syndromic forms and eligibility for precision therapies. The recent success of OTOF gene therapy trials and the resultant indirect delay in cochlear implantation in some cases, underscores the importance of timely genetic diagnosis. However, access to genetic testing remains limited due to the need to travel to specialized centers, long wait times for geneticist appointments, and variable insurance coverage. Given the extreme genetic heterogeneity of hearing loss, there is a critical need for a large, nationwide natural history study to define genotype–phenotype correlations, enable collaborations worldwide to improve rare variant classification and genetic diagnosis, and leverage these insights to improve clinical outcomes. The HearGene Connect Registry and Natural History Protocol is designed to collect genetic and phenotypic data over many years from 10,000 individuals with hearing loss and their close family members from all over the United States and its territories. Individuals of any age are eligible to participate if they experienced hearing loss before the age of eight years. Participants will be asked to share health and audiological information via questionnaires, upload available audiograms and provide access to their temporal bone images. When a genetic diagnosis is available, variant pathogenicity will be confirmed based on current knowledge and integrated into the registry. If the etiology is unknown, biospecimens will be collected for genetic analysis. Genetic test results will be shared with participants through an educational session and written clinical reports. Certified genetic counselors will be available to answer questions. Participants will be re-contacted annually to update health and hearing information, enabling longitudinal analyses.

Collected data will be used to develop gene- and variant-specific phenotype profiles, that together with educational material will help participants better understand their hearing loss. Participants will also receive information on relevant FDA-approved interventions (e.g., cochlear implantation) and potential eligibility for emerging clinical trials, supporting their informed and timely choices on therapeutic interventions. Deidentified genotype–phenotype data will be made available in an open-access platform, enabling clinicians and genetic counselors to provide evidence-based prognostic guidance and care recommendations, and supporting researchers and industry partners in designing future clinical trials and therapies. HearGene Connect gnomAD will provide participant aggregated genomic data to facilitate global collaborations, variant classification, and reverse phenotyping. As HearGene Connect expands, summary statistics and gene- or variant-level information will be publicly shared to deepen understanding of hearing loss in the United States population. These resources will empower participants with greater knowledge of their diagnosis, prognosis, and therapeutic options; and offer them opportunities to engage in future research and treatment studies. They will also assist clinicians in counseling and management; and enable discovery of new gene–phenotype relationships.

Poster Session I With Coffee

1:00 p.m. - 2:30 p.m.

Ballroom A, Floor 3

Due to the policies of the Puerto Rico Convention Center, the Poster Hall will be available to attendees between the hours of 7AM – 11PM (local time), with a 1- hour break every day between 11AM and 12 PM (Noon) to facilitate setup for the next poster session. This is an appreciated extension of their normal permitted hours. ePosters are available on the conference website and the ARO MWM mobile app.

SA1. Immune Response in the Human Macula Utricle and Sacculle After Cochlear Implantation

Ji Eun Choi¹, Shinya Ohira², Ivan Lopez³, Gail Ishiyama³, Akira Ishiyama³

¹*Dankook University*, ²*St. Marianna University School of Medicine*, ³*David Geffen School of Medicine at UCLA*

Background: An inflammatory foreign body response has been proposed to mediate the development of fibrosis and new bone formation following cochlear implantation (CI). Prior human temporal bone studies have demonstrated an increase in macrophage presence in the cochlea following CI but few prior studies have evaluated these factors in the vestibular system. Fibrosis in the vestibular system following CI has been proposed to mediate hydrops formation associated with vertigo. This study investigates the immune response in human macula utricle and sacculle following CI in the human temporal bone.

Methods: Archival human temporal bones from six patients with a history of CI, and the non-implanted contralateral side (Age range 65-92), and 3 normal controls with normal hearing (Age 26, 38, 50 years old) were studied. Celloidin sections containing the macula utricle and sacculle underwent immunofluorescence (IF) using antibodies against the following macrophage markers: Iba1, CD68, CD163, and CD206. Colocalization of the macrophage markers was achieved using laser confocal microscopy.

Results: The maculae sacculle and utricle of the CI implanted side showed an abundant presence of Iba1-IF and CD163-IF cells. A less abundant presence of CD68-IF and CD206-IF macrophages was demonstrated. Iba1-IF cells and CD163-IF cells were present in the stromal tissue underneath the vestibular epithelium and in the basal sensory epithelium. CD68-IF and CD206-IF cells were present predominantly in the stromal tissue. In the contralateral non-CI cochlea and controls, there was a minimum presence of cells expressing any of the macrophage markers.

Conclusions: There was an abundance of macrophages in the human macula sacculle and utricle in the implanted side but not in the contralateral non-implanted side nor in the normal hearing controls. This supports the presence of inflammatory and anti-inflammatory processes in the vestibular system similar to the processes we have previously demonstrated in the cochlea following cochlear implantation.

SA2. The Mechanism Analysis on Cochlear Supporting Cell Activation in Response to Viral Infection Using RNA Sequencing

Yushi Hayashi*¹

¹*Nippon Medical School*

Background: Sudden sensorineural hearing loss (SSNHL) is a relatively frequent otologic disorder observed worldwide, characterized by acute unilateral sensorineural hearing loss. Viral infection has been proposed as one possible etiology; however, the underlying mechanisms remain unclear, and therefore no fundamental treatment has yet been established. We previously reported that cochlear supporting cells (SCs) are key components of immune responses to viral infection in the organ of Corti. In the early phase of infection, SCs protect cochlear hair cells (HCs) by secreting type I interferons (IFNs) via the retinoic acid-inducible gene I (RIG-I)-like receptor (RLR) signaling pathway. In the late phase, however, SCs promote HC necroptosis by secreting TNF-related apoptosis-inducing ligand (TRAIL). Intriguingly, SCs also express F4/80, a canonical macrophage marker, highlighting their unexpected immune-like properties. Given their role as glial-like cells, the response of SCs can be considered a form of glial activation; however, SCs are of ectodermal origin, unlike microglia, which are mesoderm-derived. In this project, we focus on elucidating the mechanisms that confer immune functions on these ectoderm-derived SCs.

Methods: 1. Organs of Corti were dissected from Ms4a3-Cre;tdTomato mice at postnatal day 2 (P2), in which monocyte-derived macrophages are labeled with tdTomato, for histological analysis.

2. Organs of Corti were dissected from CD1 mice at P2 for tissue culture. After overnight incubation, tissues were infected with Theiler's murine encephalomyelitis virus (TMEV) at 3.0×10^7 pfu/ml. At designated time points, virus-infected and mock-treated organs of Corti were dissociated, stained for F4/80, and subjected to FACS sorting. F4/80-positive cells were collected from each group for RNA sequencing.

Results: 1. No tdTomato-positive cells were detected among SCs or within the SC region, indicating that SCs are of ectodermal origin and do not include monocyte-derived macrophages.

2. Compared with mock-treated organs of Corti, TMEV-infected organs of Corti contained significantly more F4/80-positive cells. Because this experimental system is *ex vivo*—where systemic macrophages cannot infiltrate the organ of Corti as they would in *vivo*—this finding supports the notion that SCs themselves upregulate F4/80 expression in response to viral infection. RNA sequencing further revealed activation of signaling pathways potentially regulating this mechanism.

Conclusions: We previously demonstrated that SCs exerting immune functions express F4/80 in virus-infected organs of Corti, as shown by immunostaining. Experiments with Ms4a3-Cre;tdTomato mice confirmed that this cell population does not include monocyte-derived macrophages. In addition, FACS analysis revealed a significant increase in F4/80-positive cells, directly reflecting SC upregulation of F4/80 in response to viral infection. We are currently conducting inhibitor assays to pharmacologically disrupt the signaling pathways identified by RNA sequencing.

SA3. Age-Related Hearing Loss and Inflammatory Profile in the Human Aged Cochlea

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Background: Age-related hearing loss (ARHL) is among the most prevalent manifestations of ageing. According to the World Health Organization, hearing loss affects ~30% of individuals over 60 years, and by 2050 one in ten people is projected to suffer from disabling hearing loss. Beyond communication impairment, ARHL accelerates cognitive decline and imposes a substantial burden on healthcare systems. Chronic low-grade inflammation, commonly referred to as “inflammaging,” has emerged as a key contributor to sensorineural hearing loss alongside environmental insults such as noise exposure. A potential player in this process may be the nucleotide-binding domain, leucine-rich repeat, and pyrin domain-containing protein 3 (NLRP3) inflammasome, a cytosolic multiprotein complex that mediates the release of pro-inflammatory cytokines, including IL-1 β and IL-18. NLRP3 activation has been implicated in neurodegenerative and cardiovascular diseases, and recent evidence links it to auditory dysfunction.

This study investigates age-related inflammatory responses in rodent models of hearing loss and human cochlear tissue, with a focus on NLRP3 inflammasome activation and its correlation with auditory decline.

Methods: Immunohistochemistry was employed to examine immune activation and inflammatory markers in rodent cochleae of different ages. These findings were systematically compared with human cochlear specimens from elderly donors. Quantitative analyses assessed microglial activation, macrophage distribution, and their association with auditory brainstem response (ABR) thresholds in rodents and spiral ganglion neuron (SGN) integrity in humans.

Results: Inflammatory activity increased with age in both rodent and human cochleae. Microglial activation patterns showed a significant correlation with ABR thresholds in rodents, while in human samples macrophage distribution was closely associated with SGN degeneration. These findings indicate a conserved age-dependent inflammatory phenotype across species.

Conclusions: Characterizing the role of inflammation in cochlear ageing advances our understanding of ARHL pathophysiology. Elucidating NLRP3-mediated mechanisms may reveal novel therapeutic targets to mitigate auditory decline and its broader neurocognitive consequences in ageing populations.

SA4. Classical Complement Pathway Activation and C3 Opsonization of Inner Hair Cell Ribbon Synapses Following Noise-Induced Cochlear Synaptopathy

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Background: Noise-induced cochlear synaptopathy (NICS) refers to the rapid and primary loss of inner hair cell (IHC) ribbon synapses following exposure to mild to moderate noise, which can occur without immediate hair cell loss or elevation on hearing thresholds. Such

synaptic loss contributes to deficits in auditory acuity, particularly in noisy environments, and may be associated with perceptual disorders like tinnitus and hyperacusis and cognitive decline. Our recent work suggested the critical role of macrophages, the innate immune cells, in facilitating the restoration of IHC ribbon synapses following NICS. To determine the factors that regulate macrophage-dependent synaptic repair, proteomics and functional enrichment analysis after NICS was performed, which revealed an upregulation of classical complement protein, C1q implicated in CNS synapse remodeling. The goal of this study was to validate the proteomics data and determine the expression, activation, source, and localization of complement proteins C1q and downstream C3 in the cochlea after noise-induced synaptopathy.

Methods: C57BL/6J mice at 5-6 weeks of age were exposed for 2 hours to a synaptopathic noise level of 93 dB SPL at an octave band of 8-16 kHz. Age-matched sham-exposed mice served as controls. C1q and C3 expression, activation, and localization in the cochlea were examined by immunoblotting, enzyme-linked immunosorbent assay (ELISA), and fluorescent immunohistochemistry, respectively, at 1-, 3-, 7-, and 15-days post-noise exposure (DPNE). The immunolabeled cochlear tissues were imaged using Zeiss LSM confocal laser and Airy scan microscopy.

Results: Complement protein C3 activity increased in the cochlea by 1 DPNE (9.9 %), peaked at 3 DPNE (60.3%) and started to decrease by 7 DPNE (33.5 %) and 15 DPNE (27.6 %) as measured by ELISA. Whereas immunoblotting for C1q and C3 showed no significant difference in their protein expression at any recovery period after noise exposure. At 1 DPNE, C3 immunolabeling showed increased fluorescent intensity (~2 fold) in the sensory epithelium of the middle region of the cochlea when compared to the sham-exposed ears. Super-resolution Airyscan imaging further demonstrated that accumulated C3 opsonizes the noise-damaged IHC ribbon synapses. In addition, macrophages displayed immunoreactivity for the complement receptor, CR3, and the phagocytic marker, CD68, in the synaptopathic noise-damaged cochlea. Lastly, in comparison to the presence of macrophages, the C3 protein levels were persistently elevated in the absence of macrophages in the sham- and noise-damaged cochlea.

Conclusions: These preliminary findings implicate classical complement pathway activation and C3 opsonization of IHC synapses in NICS, and that macrophages regulate C3 levels in the damaged cochlea. Future studies will determine the source of C3, macrophage engulfment of complement-tagged synapses, and the obligatory roles for C1q, C3, and CR3 in macrophage-dependent ribbon synaptic repair after NICS.

SA5. Glucocorticoid Receptor Beta Expression as a Marker of Steroid Resistance in Autoimmune Inner Ear Disease

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Background: Autoimmune inner ear disease (AIED) is a form of sensorineural hearing loss that is characterized by either fluctuation or worsening hearing loss in either one or both ears. The first line of treatment for AIED is high-dose corticosteroids. While 70% of initially respond, many lose responsiveness over time. Steroids are the mainstay of treatments as previous trials of other immunosuppressants, such as methotrexate and TNF-alpha inhibitors, have not shown significant benefit. Patients who fail to respond to corticosteroids express high levels of IL-1 β (Pathak et al 2011), and an early-stage clinical trial shows that anakinra, a recombinant IL-1 receptor antagonist, improves hearing capabilities in 46-70% of

corticosteroid-resistant AIED patients (Vambutas et al 2014, Vambutas et al, 2025). The mechanism(s) underlying this steroid resistance remain poorly understood. Evidence from other steroid-resistant inflammatory diseases such as asthma and ulcerative colitis suggests the involvement of glucocorticoid receptor beta (GR- β). Glucocorticoid receptor alpha (GR- α) is the active glucocorticoid receptor isoform that mediates anti-inflammatory effects, while GR- β is a splice variant that does not bind glucocorticoids and inhibits GR- α 's transcriptional activity, contributing to steroid resistance. The role of GR- β has not been studied in AIED.

Methods: We examined the relative mRNA expression and protein levels of GR- β and GR- α in peripheral blood mononuclear cells (PBMCs) of patients with AIED using qRT-PCR and Western blot, and investigated how these levels change following treatment with dexamethasone and anakinra under inflammatory conditions.

Results: In PBMCs from corticosteroid-resistant AIED patients, the relative expression of GR- β appears to increase with dexamethasone treatment when co-stimulated with LPS, while treating with anakinra and LPS reduces GR- β expression. Furthermore, this change was also reflected when assessing intracellular GR- β protein with Western blotting.

Conclusions: Characterizing the role of GR- β expression can deepen our understanding of steroid resistance in AIED patients and better predict who may respond to steroids. Further studies are underway to characterize the effect of interleukin-1 expression on GR- β .

SA6. Distinguishing Neurons in the Central Nucleus and Cortex of the Inferior Colliculus by Multiparametric Classification of Pure-Tone Responses

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Background: The inferior colliculus (IC) is a central hub of auditory processing in the midbrain, integrating ascending and descending inputs arising from the auditory pathway as well as non-auditory information originating from other brain centers. These inputs target distinct subdivisions of the IC. Ascending inputs primarily innervate neurons in the central nucleus of IC (CNIC), whereas descending and multimodal inputs target the cortex of the IC (CtxIC). This differential innervation pattern suggests that the functions of these subdivisions will also be fundamentally distinct. However, the response properties of single neurons from these subdivisions to auditory stimuli are largely similar, and while some tuning differences have been reported, classifying IC subunits based on these differences has remained challenging. In this study, we examined whether neurons could be reliably localized to the CNIC and CtxIC using only response properties in both awake and isoflurane-anesthetized mice.

Methods: We used pure-tone responses to construct the frequency response areas of neurons localized to known IC subdivisions, and extracted tuning parameters (e.g., threshold, bandwidth, etc.) as well as response features (e.g., spontaneous rate and driven rate).

Results: Individual features failed to discriminate neurons from CNIC and CtxIC in awake or anesthetized states ($d' < 1$). However, a machine learning classifier (random forest) using a combination of the features could localize neurons to the CNIC or CtxIC at the highest accuracy ($d' > 1$).

Conclusions: These results demonstrate that CNIC and CtxIC responses differ subtly along multiple parameters, and combining these differences using appropriate multi-parametric

analytical techniques is necessary to effectively classify and localize single-neuron recordings to different IC subunits in awake or anesthetized in vivo studies.

SA7. The Influence of Ascending T-Stellate Projections on Sound Responses in the Auditory Midbrain

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Background: The inferior colliculus (IC) is a critical hub of computation in the central auditory pathway. Nearly all ascending projections from the auditory brainstem converge at the IC, and the IC is crucial for many aspects of sound processing. One major source of ascending input from the lower brainstem to the IC comes from T-stellate neurons in the ventral cochlear nucleus (VCN). T-stellate neurons encode information about sound frequency and intensity, including rapid fluctuations in sound intensity that are important for speech processing. T-stellates also represent the only class of neurons within the VCN that provide direct input to the IC, but how that input contributes to sound processing in the IC in vivo remains largely unknown. Recent work in our lab revealed that T-stellate input onto IC neurons undergoes short-term synaptic plasticity in vitro. Thus, we hypothesize that inhibiting T-stellate input to the IC reduces the frequency and amplitude modulation selectivity of many IC neurons, while also reducing the adaptation of IC neuron firing during ongoing sounds.

Methods: Using multi-channel silicon probe recordings and optogenetic techniques, we are investigating how silencing T-stellate input to the IC influences how IC neurons respond to sounds in awake, head-fixed mice. To specifically and reversibly inhibit T-stellate projections, we inject an adeno-associated virus encoding the inhibitory opsin eOPN3 into the VCN of mice. Two to six weeks later, we use a 32-channel probe with an integrated optical fiber to record neuronal spiking in the IC while locally delivering light on alternating trials to selectively activate eOPN3 in T-stellate synapses. During recordings, we are examining responses to tone bursts and sinusoidally amplitude modulated noise and tones to determine how the frequency tuning and amplitude modulation selectivity of IC units changes between control conditions and when local T-stellate input is inhibited. We are also comparing the spike-frequency adaptation that occurs during tone bursts between control and light-on conditions.

Results: We have optimized the surgical preparation and virus injections. We are now collecting data to determine how IC neuron responses change between control conditions and when T-stellate inputs are optogenetically inhibited.

Conclusions: The expected results of this study will provide insights on how T-stellate input contributes to frequency tuning, amplitude modulation selectivity, and adaptation in the IC.

SA8. Sex- And Etiology-Specific Effects on Predictive Processing in the Inferior Colliculus of Two Rat Models of Autism

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Background: Atypical sensory processing is a core feature of autism, manifesting in a context-dependent manner that becomes more pronounced as environmental complexity increases. Predictive coding theory provides a unifying framework for these sensory differences and their heterogeneity across sex and etiology. According to this account, mental models of the environment, which typically anticipate incoming sensory stimuli and support efficient brain function, diverge in autism, resulting in less flexible models that struggle to accommodate changing and dynamic situations.

Predictive processes operate within the brain's hierarchical architecture, where higher- and lower-order regions interact in a feedback loop: top-down predictions are generated, and bottom-up prediction errors signal mismatches between mental models and the environment. The neural mechanisms underlying these divergences at the subcortical level, as well as their modulation by sex and etiological origin, remain poorly understood.

Methods: We investigated spontaneous activity and contextual auditory processing at the single-neuron level in the inferior colliculus, a midbrain hub that serves as an early site for contextual sound encoding. Using two rat models of autism—one carrying a heterozygous deletion of *GRIN2B* and another prenatally exposed to valproic acid, representing genetic and environmental etiologies—we recorded auditory responses from lemniscal and non-lemniscal subdivisions during oddball and cascade paradigms that dissociate adaptation from deviance detection.

From these responses, we derived three indices of predictive processing: repetition suppression, reflecting neural adaptation to repetitive stimulation; prediction error, the response to violations of established regularities that drive mental model updating; and neuronal mismatch, which integrates both adaptation and deviance detection to capture the overall mismatch response.

Results: Both autism-like models exhibited disrupted spontaneous firing and predictive processing in a sex- and region-specific manner, with the non-lemniscal inferior colliculus emerging as the primary locus of dysfunction. Males across autism-like models displayed reduced repetition suppression and attenuated mismatch responses, whereas females showed enhanced suppression and elevated mismatch signals relative to controls.

Conclusions: These findings identify convergent yet sex-dependent disruptions in subcortical predictive coding across distinct autism etiologies and establish a framework for exploring sensory heterogeneity in neurodevelopmental disorders.

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SA9. Identifying Key Characteristics of Two New Neuron Types in the Inferior Colliculus

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Background: Uncovering how individual neurons contribute to circuit-level computations relies heavily on our ability to distinguish and selectively manipulate individual neuron types. The inferior colliculus (IC), the neuron-dense midbrain hub of the central auditory pathway, poses challenges in this regard, making it difficult to distinguish the roles of individual

neurons in auditory computations. However, recent advances have enabled the identification of subpopulations of IC neurons using molecular markers such as vasoactive intestinal peptide (VIP) and neuropeptide Y (NPY). Here, we report the discovery of two novel molecular markers, secreted phosphoprotein 1 (SPP1) and prodynorphin (PDYN). We have identified fundamental physiological, anatomical and morphological features of SPP1 and PDYN IC neuron types.

Methods: To characterize the intrinsic physiological properties of PDYN and SPP1 neurons, we used *Pdyn-IRES-Cre* and *Spp1-IRES-Cre* mice crossed with an *Ai14* reporter line to perform whole-cell patch clamp recordings in brain slices targeted to each neuron type. To determine the anatomical distributions of PDYN and SPP1 neurons in IC, we conducted fluorescent in situ hybridization (FISH) on IC sections from C57BL/6J and B6.CAST-Cdh23Ahl⁺ mice. To uncover morphological patterns in PDYN and SPP1 neurons, we crossed their respective Cre lines with MORF3 mice that sparsely label Cre-expressing cells with a multi-V5-epitope reporter protein. We extracted the IC and conducted whole tissue clearing, immunolabeling, light-sheet microscopy, and 3-D anatomical reconstructions of individual somas, dendrites, and axons.

Results: We find that *Pdyn* mRNA labels a population of excitatory neurons that are distinct from previously identified IC markers and that *Pdyn*⁺ neurons are present throughout the major subdivisions but are at higher densities in the IC dorsal cortex. Furthermore, our findings suggest that *Spp1* mRNA labels two populations of IC neurons: excitatory neurons that are distinct from previously identified excitatory neuron types, and inhibitory neurons. Using whole-cell patch clamp recordings we find that PDYN neurons have consistent intrinsic physiological properties, including sustained firing patterns in response to current steps while SPP1 neurons exhibit both sustained and adapting firing patterns in response to current steps, suggesting two subtypes that we hypothesize correlate with the excitatory and inhibitory subtypes of SPP1 neurons. Using sparse labeling and light-sheet microscopy, we are generating high-throughput 3-D reconstructions of SPP1 and PDYN neurons in the intact IC to identify patterns in their dendritic shape, soma location, and axonal projections.

Conclusions: Our findings suggest that SPP1 and PDYN are strong candidates for markers of novel IC neuron populations that do not overlap with previously identified markers for IC neurons. We also developed a high-throughput approach to investigate the 3-D morphology and axonal projections of molecularly defined IC neuron types utilizing whole IC tissue clearing and light-sheet microscopy. We are currently using quantitative methods to determine the axonal projection tendencies of each neuron type.

SA10. Pupil-Linked Modulation of Noise Correlations and Auditory Information Coding in the Inferior Colliculus

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Background: Neuromodulatory brain state, indexed by pupil size, strongly shapes sound-evoked responses in the auditory thalamus and cortex (McGinley et al., 2015). In particular, response gain tends to exhibit an inverted-U relationship with pupil size on average. Yet it is still uncertain at which levels of the auditory pathway state-dependent modulatory signals shape sensory processing, and how they affect encoding of auditory information in earlier structures like the midbrain.

Methods: To address this, we assessed how brain state influences sensory processing in the inferior colliculus (IC) by simultaneously measuring pupil size and neural activity in head-fixed, awake mice. Using Neuropixels probes, we recorded central IC (ICC) responses to pure tones across 17 sessions in 10 animals. Computational analyses included pairwise noise correlations within and between neuronal subpopulations, stimulus decoding at both pairwise and population levels with decorrelation procedures to evaluate how noise correlations shape information coding. As a parallel control, we also implemented a pseudo-pupil size analysis to test whether the observed effects truly reflected state dependency.

Results: Our analyses revealed pronounced state dependence in ICC activity. At the population level, response gain exhibited an inverted-U pattern. In addition, we identified distinct neuronal subpopulations: while the largest group showed the inverted-U profile, others displayed either monotonically decreasing or monotonically increasing activity with arousal. Extending beyond response gain, we investigated how arousal-dependent modulation shapes population coding and influences auditory information represented in IC activity. We first measured decoding accuracy across pupil-defined states and observed systematic differences in discriminability that varied both with state and across neuronal subpopulations exhibiting distinct pupil-linked modulation patterns. Given these state-dependent changes in decoding, we further analyzed how brain state influences the magnitude and pattern of noise correlations, both within and across neuronal subpopulations. In addition, we examined the extent to which these correlations could enhance or limit the information available in the population, and how this impact is shaped by state-dependent modulation.

Conclusions: Our current results highlight the IC as an early auditory stage where brain state modulates response gain and population-level coding, and that noise correlation is state dependent. Ongoing analyses will determine how state-dependent noise correlations among IC neurons contribute to the representation of sensory information.

Reference: McGinley, M. J., David, S. V., and McCormick, D. A. (2015). Cortical membrane potential signature of optimal states for sensory signal detection. *Neuron*, 87(1), 179–192. doi:10.1016/j.neuron.2015.05.038

SA11. Expression of Mitochondrial Calcium-Regulatory Genes in Cochlear Aging

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Background: Age-related hearing loss (ARHL) contributes to social isolation, reduced independence, anxiety, depression, cognitive decline, and increasing healthcare costs. The condition is primarily associated with degeneration and death of mitochondria-rich cochlear cells, including hair cells, spiral ganglion neurons, and cells of the stria vascularis. Although extensively studied, the exact molecular mechanisms underlying cochlear aging and cell death remain poorly defined. Evidence suggests that programmed changes in gene expression, cumulative environmental insults, e.g., noise, ototoxins, as well as genetic predisposition all contribute to ARHL. Our previous findings indicate that dysregulated mitochondrial calcium homeostasis accelerates ARHL progression. We hypothesize that age-dependent alterations in the expression of mitochondrial calcium-regulatory genes impair energy metabolism, promoting cochlear cell loss and hearing decline. To test this hypothesis,

we examined age-related expression dynamics of genes involved in mitochondrial calcium and energy metabolism, such as *Mcu*, *Micu1*, *Micu2*, *Mcur1*, *Emre*, and *Mcub*, in cochlear tissue from FVB/NJ mice at multiple points across the aging timeline.

Methods: Gene expression was quantified using real-time quantitative PCR (RT-qPCR). Cochlear tissues were collected from FVB/NJ mice at 1, 6, 9, and 12 months of age. Total RNA was isolated with the Qiagen RNeasy kit and reverse-transcribed to cDNA using the qScript™ cDNA synthesis kit. Gene-specific primers and PowerUp™ SYBR™ Green Master Mix were used for amplification, and Ct values were analyzed. Data quality control was performed with DataAssist™, while fold changes were calculated using the $2^{-\Delta\Delta C_t}$ method in Microsoft Excel. Statistical analyses were conducted with GraphPad Prism 10. To validate differentially expressed candidates, results were compared with previously reported single-cell RNA sequencing (scRNA-seq) datasets exhibiting hair-cell degeneration.

Results: Of the 30 genes examined, several, including *Otof*, *Cav1*, *Gjb2*, *Mcub*, and *Serca2*, displayed significant expression changes associated with hair-cell degeneration in both mouse cochlear tissues and human utricle dataset. Interestingly, *Cdh23* expression was significantly elevated in RT-qPCR data but remained unchanged in the scRNA-seq dataset. *Micu1* and *Micu2* were upregulated in the supporting cell cluster in the scRNA-seq dataset but not in the hair-cell cluster or mouse RT-qPCR results.

Conclusions: Age-related alterations in cochlear gene expression may disrupt mitochondrial calcium homeostasis and energy metabolism leading to cochlear cell death and progression of ARHL. The next step will be to elucidate the key molecular mechanisms underlying ARHL and to identify potential therapeutic targets.

SA12. Aging and Noise Exposure Cause Differential Patterns of Cochlear Synaptopathy and Central Gain

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Background: Adults of all ages experience a wide variety of potentially damaging recreational and occupational acoustic exposures. These noise exposures may cause a loss of cochlear afferent synapses that remain “hidden” from audiometric assessments. Aging results in a progressive loss of cochlear synapses, while early noise exposures exacerbate cochlear synapse loss. However, the vulnerability to cochlear synapse loss, and subsequent effects on the central auditory pathway at later ages are still unclear. In this study, we investigate the interactions between aging and acoustic overexposures, focusing on cochlear deafferentation and subsequent central gain in a mouse model (CBA/CaJ). By systematically varying age of noise exposure, we aim to investigate vulnerabilities to noise-induced cochlear synapse loss and central gain as a function of age.

Methods: Male and female CBA/CaJ mice (N = 54) were exposed to a single synaptopathic noise exposure (100dB SPL, 2 hours, 8-16kHz) at various time points across their lifespan. Testing occurred two weeks post. Results were compared to unexposed age-matched litter-mate controls. Electrophysiological assessment of cochlear synapse loss and central gain were made using EEG-based envelope following responses (EFRs). Histological assessment of cochlear synapse loss was made using immunostaining of presynaptic ribbon terminals and post-synaptic glutamate receptors in the inner hair cells.

Results: Noise exposure produced significant synapse loss at the 30 kHz region while largely sparing the 12 kHz region across exposure ages, confirming the tonotopic vulnerability of noise-induced synaptopathy. Early noise exposure resulted in significant cochlear synapse loss and increased central gain compared to age-matched controls. The degree of synapse loss due to noise exposure was the same, regardless of age at noise exposure, suggesting a floor effect on the amount of cochlear deafferentation. However, noise exposure at later middle-ages resulted in lesser central gain, despite similar degrees of deafferentation.

Conclusions: Our results suggest that age at noise exposure can have a dramatic effect on the compensatory ability of the auditory system when faced with decreased peripheral inputs. These unique profiles of cochlear synapse loss and central gain likely have differing effects on speech perception in noisy conditions. Clinically, these results suggest that an individual's age when exposed to noise should be considered in determining potential damage to the auditory system and effects. The electrophysiology measures used here could potentially lead to objective clinical biomarkers for cochlear synapse loss and central gain assessments in the clinic.

SA13. Cortical Auditory Encoding Deficits in Mild Cognitive Impairment With and Without Alzheimer's Disease Biomarkers

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Background: Hearing loss is a leading potentially modifiable risk factor for dementias such as Alzheimer's disease (AD), yet auditory dysfunction in early AD can occur even in individuals with clinically normal audiometric thresholds. A recent meta-analysis concluded that auditory EEG may help identify individuals at risk for developing AD (Tarawneh et al., 2024). Specifically, AD is associated with longer latencies for N200 (sensory encoding) and P300 (cortical processing) compared to healthy controls. While no significant latency differences were observed between mild cognitive impairment (MCI) and AD groups, prior studies did not test for AD biomarkers, leaving it unclear whether auditory processing deficits in MCI reflect AD-specific pathology. Furthermore, some longitudinal work suggests that individuals with MCI who progressed to probable AD within five years showed significantly longer P300 latencies than those who did not (Golob, Irimajiri, and Starr, 2007).

Methods: In the present study, we recruited individuals with MCI who underwent AD biomarker testing, along with cognitively healthy controls. EEG data were recorded during a two-tone auditory oddball task. Each block consisted of 120 standard tones (500 Hz) and 30 deviants (1500 Hz) presented in a pseudo-randomized sequence at 85 dB SPL. Tones were 100 ms in duration and separated by 1500-1800 ms (jittered IOI). Each block began with at least five standards, and each deviant was followed by at least three standards before another deviant occurred. Participants were instructed to attend to the tones. Ten times per block (on five standards and five deviants), the tone stream paused and participants reported whether the last tone was "high" or "low", with a minimum of eight tones between prompts. Unlike traditional oddball tasks, this design minimized working memory demands ("count the deviants") and reduced motor artifacts from button presses ("press when you hear a deviant").

Results: We hypothesize that individuals with MCI and AD biomarkers (MCI+AD) will show delayed N200 and P300 responses relative to MCI without AD biomarkers (MCI-AD) and healthy controls, reflecting deficits in both sensory encoding and cortical attention/memory processes, respectively. Our target sample size is N = 60 (n = 20 per group), and preliminary data collection is underway.

Conclusions: This study aims to determine whether cortical auditory processing deficits in MCI are uniquely linked to AD-related neurodegeneration or reflect broader cognitive impairment. If confirmed, auditory ERPs measures could provide sensitive, scalable tools for early detection and monitoring of AD progression. Moreover, distinguishing MCI(+AD) from MCI(-AD) may improve diagnostic precision and inform earlier, targeted interventions. Preliminary results will be discussed. [Supported by NIH/NIA Michigan Alzheimer's Disease Research Center grant P30AG072931, the University of Michigan Alzheimer's Disease Center (Berger Endowment), and NIH grant R21DC019409.]

SA14. Microglia Interact With Gabaergic Interneurons and Myelin in the Central Auditory System of Aged Mice

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Background: Aging is a complex disorder with multiple pathologies in the central auditory system, including chronic inflammation that leads to an increased number of pro-inflammatory microglia, the loss of GABAergic inhibition (often resulting in hyperexcitability), and reduced myelin integrity. Both decreased GABAergic inhibition and reduced myelin integrity are associated with age-related auditory deficits. There is increasing evidence that pro-inflammatory microglia play an important role in both the modulation and maintenance of GABAergic inhibition as well as myelin maintenance. However, the role of pro-inflammatory microglia in age-related central auditory system deficits is unknown. Here, we examine age-dependent alterations in the numbers of the of pro-inflammatory microglia and GABAergic interneurons, myelin state, and interactions of microglia with interneuron and myelin structures in the mouse auditory brainstem and cortex.

Methods: Quantitative immunohistochemistry was performed on sections of the auditory cortex (AC) and the central nucleus of the inferior colliculus (ICC) in young adult (4M) and aged (2+ Y) CBA/CaJ mice to characterize the relationship between activated microglia with loss of GABAergic interneurons and myelin integrity with age. GABAergic neurons were identified using GAD65/67 or GAD67 with NeuN co-expression. Activated, pro-inflammatory microglia were determined by morphological changes in IBA1+ cells. Myelin basic protein (MBP) was used to identify myelin structures. Analysis was performed using z-stack confocal images to quantify the number of GABAergic interneurons and microglia, and the interactions of microglia with GABAergic interneurons and MBP+ myelin structures. To identify expression-level changes in genes that occur with aging, we performed bulk RNA sequencing analysis of the AC and IC tissues.

Results: Aging mice exhibited fewer GAD65/67+ and GAD67+ neurons in both the ICC and AC compared to young mice, along with reduced MBP expression in both regions. Aged mice also exhibited a greater number of IBA1+ cells, with a greater proportion of ramified cells, in the ICC and AC compared to younger mice, although overall more IBA1+ cells were found in the AC. Interestingly, we found a subset of aged IBA1+ cells in only in the AC that exhibited engulfment of GAD67+ cell processes or debris. Enrichment analysis of RNA sequencing data revealed that aging resulted in more dysregulation of myelin in the aged AC compared to the aged IC.

Conclusions: These results show that aging results in increased numbers of activated microglial throughout the central auditory system and that this increase is coupled with a reduction in GABAergic interneurons and reduced myelination. These data suggest that aging, pro-inflammatory microglia may play a role in age-related structural changes in the

central auditory system, although further studies directly associating microglial activity with GABAergic interneuron activity and myelin are needed to investigate any causal relationships.

SA15. Aging Masks Auditory Dysfunction Associated With the Autism Spectrum Disorder Gene Mef2c

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Background: Autism spectrum disorders (ASDs) are associated with sensory deficits. Specifically, difficulties in hearing and sound processing are often reported in both children and adults with ASD. Mutations or deletions in the MEF2C gene results in a syndromic form of ASD known as MEF2C haploinsufficiency syndrome (MCHS). Global Mef2c heterozygosity is sufficient to elicit MCHS-like phenotypes in mice, including sensory deficits. Young-adult Mef2c-heterozygous mice exhibit auditory nerve functional impairment and a moderate reduction in hearing sensitivity. To date, most studies of auditory dysfunction in ASD preclinical models have focused on early development and young adulthood. The long-term trajectory of these deficits with advanced age is largely unknown, highlighting a critical gap in understanding the intersection of aging and auditory function in ASD.

Methods: Global Mef2c-heterozygous mice were backcrossed to B6.CAST mice to eliminate the Cdh23Ahl mutation present in the C57BL/6J background, which causes early-onset hearing loss, for auditory functional assessments. Auditory brainstem responses were used to assess hearing sensitivity and auditory nerve activity in aging Mef2c-heterozygous and wildtype mice. Bulk RNA-sequencing of cochlear tissue and Simoa (Quanterix) analysis of peripheral blood were performed to assess differential gene expression and circulating neurodegenerative biomarkers, respectively. Cognitive and behavioral phenotypes relevant to MCHS were evaluated using open field, vertical locomotor, social interaction, and acoustic startle tests.

Results: Aging Mef2c-heterozygous mice displayed similar auditory thresholds to wildtype controls, suggesting similar age-related hearing decline. Analysis of suprathreshold auditory brainstem responses revealed no significant differences in wave I amplitude or latency between groups, indicating that both genotypes experience similar age-related reductions in peripheral auditory nerve function. However, aging Mef2c-heterozygous mice exhibited a reduced acoustic startle response compared to wildtype controls, suggesting enhanced age-dependent declines in central auditory processing. Behavioral testing revealed increased hyperactivity in aging Mef2c-heterozygous mice compared to wildtype controls during the open field and locomotor activity assays, similar to changes reported in young-adult mice. Social interaction with social novelty test showed no significant differences between genotypes, indicating similar social behavior with aging. Bulk RNA-seq analysis of cochlear tissue identified a small number of differentially expressed genes between aging Mef2c-heterozygous and wildtype mice. Finally, biomarker analysis using Simoa (Quanterix) technology revealed evidence of increased age-related neurodegeneration in Mef2c-heterozygous mice compared to wildtype controls, including increased levels of neurofilament light chain (NFL).

Conclusions: While young-adult Mef2c-heterozygous mice exhibit auditory nerve impairment and mild hearing loss, age-related declines seem to mask auditory sensitivity,

peripheral auditory nerve activity, and cochlear gene expression changes between Mef2c-heterozygous mice and wildtype controls. Persistent hyperactivity and reduced acoustic startle responses, however, highlight central auditory processing effects and underscore the need to investigate how ASD risk genes influence cognitive behavioral outcomes across the lifespan.

SA16. Neurophysiological Functional Connectivity Changes During Difficult Listening in Older and Younger Adults

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Background: Older adults often report increased difficulty understanding speech in noisy listening environments. These difficulties are thought to arise from neurophysiological changes associated with aging. Speech comprehension is believed to rely on coordinated activity across distributed cortical regions, mediated by the directional flow of electrical signals – their functional connectivity. This connectivity can be analyzed within specific frequency bands, e.g., delta and theta, each thought to play a distinct role in speech and language processing.

Methods: We utilize the Network Localized Granger Causality (NLGC) framework applied to magnetoencephalography (MEG) data to simultaneously estimate neural currents in cortex and the functional connectivity network that connects them. Whole-brain connectivity graphs were analyzed using graph-cluster enhancement and permutation testing, with additional analyses focused on language-related regions of interest.

Temporal Response Functions (TRFs) were obtained by relating NLGC-derived neural current estimates to the speech stimulus presented to listeners. These TRFs enabled localization of stimulus-driven responses within encompassing age-affected networks.

Results: We elucidate how cortical circuits change as listening conditions become increasingly adverse, and we reveal differences in regional connectivity between older and younger individuals. We provide evidence for distributed network changes with aging, particular to the delta and theta bands, and demonstrate the sensitivity of these network changes to listening conditions.

Conclusions: Our findings highlight age- and condition-dependent shifts in speech-related cortical connectivity across neurophysiological frequency bands. By integrating NLGC-derived networks with TRF analysis, we localize hierarchical speech processing within these circuits. Although demonstrated on a speech-listening dataset, the approach is broadly applicable to MEG studies of neural dynamics.

SA17. Relations of Auditory Temporal Processing Tests With Other Hearing Measures in Young Adult Mice

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Background: Neurophysiological assessment in animal models has traditionally focused on peripheral integrity of the auditory system. Yet, speech-in-noise difficulties are often cited as a primary patient complaint in outpatient audiologic practice, which may involve post-cochlear processing. Thus, there is utility in measurement of temporal processing in hearing loss animal models. The auditory brainstem response gaps-in-noise test (ABR-GIN) has previously shown efficacy in this area. Previous work has focused on showing a change in ABR-GIN responses in young versus older groups of mice, or in other studies, drug effects on auditory temporal coding. In this presentation we offer a relatively novel perspective with characterization of the neurophysiological profile of the 3-month-old CBA/CaJ hearing loss mouse model in reference to the ABR-GIN. These data were collected as the first timepoint in a 2-year ongoing longitudinal study of the aging auditory system.

Methods: Our analysis included N = 24, 3-month-old CBA/CaJ mice (12 male; 12 female). Animals were anesthetized using a ketamine/xylazine mixture of 120 and 10 mg/kg to the mouse's body weight. ABR-GIN recordings were obtained with presentation of a 25-ms noise burst followed by 0, 1, 2, 4, 8, 16, 32, and 64-ms durations of silence prior to a second wideband noise burst. Thresholds were determined by identifying the shortest gap duration with a replicable response after the silence. Latency and amplitude were measured for waves I and IV for each response. All physiology measurements were obtained using TDT BioSig software. Distortion product otoacoustic emissions were measured using L1 of 65-dB SPL and L2 of 50-dB SPL with a constant frequency ratio of 1.25 for F1 and F2. Auditory brainstem response recordings were obtained for 3, 6, 12, 26, 20, 24, 32, 36, and 48 kHz and using a wideband click. Latency, amplitude, and threshold were marked. Recordings were obtained twice for each frequency for replicability.

Results: Reliable ABR-GIN responses were recorded from all 24 mice, with thresholds recorded between 1 and 4 ms. Threshold ABR responses showed elevation in the very lowest frequencies compared to the mid and high frequency ranges. Emissions were most robust in the mid frequency range in the range of best hearing for the CBA mouse, but small responses in the lowest and highest frequencies. Relationships between the different test measures are being assessed to identify potential patterns of association, and how they change with age.

Conclusions: In conjunction with other traditional neurophysiological measures, the ABR-GIN can provide useful information related to temporal processing in an age-related hearing loss mouse model. The inclusion of this measure offers a broader characterization of auditory function with peripheral through central involvement. Ongoing longitudinal analyses will further clarify how these relationships evolve with age and contribute to understanding biomechanisms underlying speech-in-noise difficulties.

SA18. How Long Does the Capacity for Synapse Regeneration Persist Following Noise Exposure?

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Background: Cochlear synapses between inner hair cells (IHCs) and spiral ganglion neurons (SGNs) are susceptible to damage caused by moderately loud noise, termed noise-induced cochlear synaptopathy (NICS), evident by histology as a loss of synapses and non-invasively by a permanent decline in auditory brainstem response (ABR) wave-I amplitude with no permanent elevation of hearing threshold. We have previously shown (Hemachandran et al. Front. Cell. Neurosci., 2024) that rolipram, a cyclic AMP PDE4 inhibitor, promotes synapse

regeneration and partial recovery of ABR wave 1 amplitude in vivo when administered one day after noise exposure. Here we ask how long the capacity for synapse regeneration persists following noise exposure. We systemically delivered rolipram either two or three days after noise exposure to determine whether rolipram retains its regenerative capacity. Establishing a therapeutic window for rolipram in recovery from NICS is critical to determining effectiveness of delayed treatment, which could enhance its potential for future clinical use in humans.

Methods: 12–14-week-old CBA/CaJ male mice were exposed to a 100 dB SPL, 8-16 kHz octave-band noise for 2 hours. ABR measures were made at 8, 16, and 32 kHz frequencies pre-noise and on post-noise day 1. Mice were subcutaneously implanted with osmotic minipumps delivering rolipram dissolved in DMSO or control DMSO on post noise day two (PND2) or three (PND3). Delivery of rolipram or vehicle control continued for two weeks (PND14), at which time ABR was measured again and the mice were euthanized. Cochleae were dissected and labeled with the following antibodies to visualize hair cells and their synapses: Myosin 6A/7 (hair cells), CtBP2 (presynaptic ribbons), and PSD-95 (postsynaptic densities, PSDs). Synapses (defined as adjacent CtBP2 and PSD puncta) were counted at 8, 16, and 32 kHz locations.

Results: Our noise exposure paradigm causes a temporary but not a permanent threshold shift, and a permanent reduction in wave-I amplitude, consistent with NICS. Rolipram itself has no effect on auditory threshold. Initiation of rolipram on PND2 partially restored ABR wave-I amplitude at 8 and 32 kHz, but not significantly at 16 kHz. Initiation of rolipram on PND3 did not restore wave-I amplitude at 16 or 32 kHz, but did partially restore wave-I amplitude at 8 kHz. Initiation of rolipram treatment on PND2 or PND3, unlike initiation on PND1, did not significantly increase synapse counts relative to vehicle control mice. Counts remained significantly lower than those of non-noise-exposed mice.

Conclusions: The capacity for rolipram to promote synapse regeneration declines rapidly after noise exposure. The therapeutic window for rolipram's effectiveness appears to be short, likely within 1-2 days post-noise exposure. Clinically, rapid intervention is critical for restoring hearing synapses after NICS.

SA19. Distinct Brain Connectivity Traits of Misophonia in Co-Existence With Hyperacusis and in Isolation

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Background: Misophonia and loudness hyperacusis are distinct sound tolerance disorders that share similar symptoms, which are involuntary and aversive responses to sounds. A comprehensive understanding of neural traits of these disorders is presently lacking. Our exploratory study aims to dissociate the intrinsic brain connectivity patterns underlying each condition which may help explain some of the variability in behavioral outcomes—such as autonomic versus emotional responses. Furthermore, we investigate neural alterations when both the disorders are endured (comorbid) so as to disentangle the unique cognitive burdens imposed by either or both conditions.

Methods: We examined resting-state functional MRI data acquired from three groups of young adults: having both misophonia and hyperacusis (MH; n=24), having misophonia only (MI; n=27), and control individuals with no complaints of decreased sound tolerance (CTR;

n=25). By training robust machine learning classification models, we processed brain-wide spontaneous functional connectivity and identified patterns that reliably differentiated either patient group from the control group.

Results: Compared to CTR, MI and MH showed atypical connectivity for the salience, somatomotor, and frontoparietal control networks. Additionally, subcortex-related connectivity patterns captured in MH classifier were also aligned with misophonia detection. Unique to MH were the connectivity trends for regions in dorsal attention network and default mode network. Interestingly, connectivity within subcortical basal ganglia was found disrupted in MI but not in MH. Taken together, distinct and overlapping resting-state neural markers of misophonia and hyperacusis warrant a more precise phenotyping and caution against conflating them in research or clinical settings.

Conclusions: Substantial alignment between the idiosyncratic network connectivity patterns of MH and MI groups indicates that core misophonia-related brain changes show dominant effects even in the presence of comorbid hyperacusis. While previous misophonia studies extensively report differences in the salience network, we identify additional connectivity dysregulations that suggest cognitive control impairments in misophonia; these likely manifest as issues with motor inhibition or attentional regulation, showcasing a previously underappreciated dimension of misophonia. Additionally, the results implicate reduced regulation of goal-directed attention, which may lead to emotional distress in presence of triggers, and psychiatric co-morbidities in the long-run. Altered subcortical connectivity trends observed in MI reflect common neural mechanisms with OCD, relating to obsessive behavioral symptoms. The impact of hyperacusis in MH is also evident through the disrupted interactions associated with higher-order visual processing and semantic integration. In summary, these connectivity traits advocate for a psychology-based therapeutic approach for misophonia. Future research should focus on translating these insights into clinical interventions such as cognitive behavioral therapy (CBT) that are grounded in the neurological and psychological characteristics of misophonia. Our results provide support for exercising CBT techniques in emotion regulation training, cognitive reappraisal, and attention-shifting strategies to positively modulate dysregulated connectivity in misophonia sufferers.

SA20. Cross-Validation of GPIAS Against an Operant Conditioning Model in the Assessment of Tinnitus in Mice

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Background: The investigation into mechanisms and pharmacological treatments of subjective tinnitus relies heavily on the development of reliable animal models. Currently, the most commonly used behavioral paradigms include operant conditioning and Gap Prepulse Inhibition of the Acoustic Startle (GPIAS). Operant models are considered to better reflect the perceptual experience of tinnitus in humans but require extensive and carefully designed training. In contrast, GPIAS allows more efficient and rapid screening of tinnitus-like behavior in animals, despite ongoing controversies regarding its theoretical foundation. Nevertheless, GPIAS remains widely applied across diverse animal models and tinnitus induction methods. Given the ongoing debate over whether GPIAS accurately detects tinnitus, this study employs a well-validated operant model to cross-validate the standard GPIAS procedure, and evaluates the sensitivity of GPIAS in detecting tinnitus induced by different etiologies.

Methods: Tinnitus was induced using either monaural noise exposure (100 dB SPL, 4–20 kHz, 2 hours) or via intraperitoneal injection of sodium salicylate (350 mg/kg). All mice underwent a two-week Sound Based Avoidance Detection (SBAD) training paradigm to establish a conditioned reflex. Those trained mice were subjected to both standardized GPIAS and SBAD tests before and after tinnitus induction. Auditory evoked potentials (AEPs) were recorded from the auditory cortex to evaluate gap detection ability. A comparative analysis of outcomes from the two behavioral assays was performed both within individual subjects and across induction methods.

Results: Both tinnitus induction methods led to significant tinnitus-like behaviors as detected in the SBAD test. The GPIAS paradigm yielded a similar proportion of tinnitus-positive animals as the SBAD test. Furthermore, cortical AEP recordings confirmed that the mice were capable of perceiving the silent gaps embedded in the GPIAS stimuli.

Conclusions: These findings support that GPIAS can serve as a sensitive screening tool for tinnitus in animal models, exhibiting comparable effectiveness to the operant conditioning paradigm. However, the validity of GPIAS does not depend on the presumed deficit in gap detection among tinnitus subjects. Tinnitus-related deficits may influence gap processing in more complex ways.

SA21. Calcium Signaling in the Organ of Corti Following Treatment of Novel $\alpha 9\alpha 10$ Nicotinic Receptor Small Molecules

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Background: Alpha9 and alpha10 subunits assemble into unique, highly Ca²⁺ permeable, nicotinic receptors (nAChRs) that are expressed in outer hair cells (OHCs) in adults and in inner hair cells (IHCs) during mouse development. Acetylcholine released from efferent terminals at the medial olivocochlear (MOC) efferents activates $\alpha 9\alpha 10$ nAChRs, creating an influx of Ca²⁺, which opens small-conductance (SK) Ca²⁺ activated K⁺ channels, hyperpolarizes hair cells, and reduces cochlear amplifier gain. Modulating $\alpha 9\alpha 10$ nAChRs can change efferent feedback and susceptibility to acoustic trauma, hearing loss, and tinnitus. In this study, we tested $\alpha 9\alpha 10$ nAChR antagonist and agonist small molecules in a cochlear explant model. We examined the specificity and mode of action of these small molecules using genetically encoded calcium indicators (GCaMP).

Methods: All procedures conformed to institutional and national animal welfare regulations and were approved by the Institutional Animal Care and Use Committee. Male and female mice of either sex were used in this study. For scale and kinetics, both GCaMP6s and GCaMP8m expressing lines were used and crossed with Gad2-IRES-Cre. Postnatal day (P) 6–30 cochlear ex vivo preparations (explants) were examined.

Cochlear Preparation

The cochlea was micro-dissected to access the sensory epithelium while preserving hair cells and supporting cells. Cochlear explants were placed in a perfusion chamber and stabilized with a harp.

Calcium Imaging, Drug Delivery, and Data Analysis

A Bruker swept-field confocal microscope was used for calcium imaging. Drug treatments included 100 μ M acetylcholine, 100 μ M muscarine, 5 μ M atropine, and 0.2–20 μ M UT-860

and UT-167. GCaMP fluorescence modulation was determined pixel-by-pixel. Statistical significance of differences was determined using Student's t-test.

Results: Quaternary salts of small molecules UT-167 (antagonist) and UT-860 (agonist) were synthesized to improve aqueous solubility. These derivatives displayed enhanced solubility for in vitro testing and pharmacological activity. Both UT-167 and UT-860 showed distinct micromolar responses in hair cells when administered via bath perfusion. In developing cochlea (P6-P8), treatment with 0.2 -6 μ M UT-860 produced large increases in Ca^{2+} in OHCs and IHCs. All three rows of OHCs responded to UT-860 with robust increases in Ca^{2+} signaling. A few distinct supporting cells also showed Ca^{2+} modulation during this developmental age. In adults (P14 and older), treatment with UT-167 showed both increases and decreases in Ca^{2+} modulation in OHCs.

Conclusions: $\alpha 9\alpha 10$ nAChRs are critical targets for continued small-molecule efforts. Detailed studies of their selectivity, kinetics, and signaling in OHCs, IHCs, and supporting cells are needed to advance this work. In parallel, in vivo bioavailability, efficacy, and safety studies are essential to determine their potential translational benefit for treating hearing loss and tinnitus.

SA22. Hyperacusis in Young Adults With Normal Hearing Does Not Differ by Autism Status

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Background: As many as 17% of the general population and 70% of autistic individuals experience some degree of hypersensitivity to sounds (i.e., hyperacusis). Compared to individuals without hyperacusis, these individuals perceive everyday sounds as abnormally loud, distressing, or even physically painful. Many sufferers adopt drastic behavioral changes to minimize exposure to sounds, often sacrificing social interactions and employment. Current tools for hyperacusis assessment lack diagnostic sensitivity and specificity, likely due, to insufficient pathophysiological understanding of the disordered processes underlying the condition in humans. Despite limited understanding of the neural underpinnings of hyperacusis, there is no evidence to suggest that hyperacusis is qualitatively or quantitatively different in neurotypical and neurodivergent populations. Thus, we propose that hyperacusis is a transdiagnostic condition that likely shares physiological mechanisms across people with and without ASD. Here, we sought to investigate the relationships between peripheral auditory function, central auditory activation, hyperacusis, and ASD. We hypothesized that individuals with hyperacusis would demonstrate signs of peripheral and central auditory dysfunction, but that their audiological profiles would not differ based on autism status.

Methods: Currently, fifty-eight adults (females = 37, mean age = 23.38, SD = 4.11) have participated; fifteen were autistic. Hearing thresholds were tested at standard- (250–8000 Hz) and extended high-frequencies (10000–16000 Hz), bilaterally. Loudness perception was characterized in all ears via loudness discomfort levels (LDLs). Ear-specific auditory brainstem responses (ABRs) were recorded using 95-, 100-, and 105 dB-SPL click stimuli and analyzed for amplitudes and latencies. Hyperacusis severity was estimated using the Hyperacusis Questionnaire (HQ). Hyperacusis status was qualified by HQ score (≥ 22) or by positive response to the question “Do you feel that you are more sensitive to sounds than other people?”.

Results: Though all participants had clinically normal hearing at standard audiometric frequencies, hyperacusis symptoms widely varied, with HQ scores ranging from 0 (no symptoms) to 37 (severe symptoms). Twenty-three participants (39.66%) met the criteria for having hyperacusis. Hyperacusis was found to be significantly correlated with lower (i.e., worse) LDLs at all frequencies ($p=0.011$). Furthermore, hyperacusis was associated with subclinical signs of peripheral auditory dysfunction; specifically, hearing thresholds were significantly poorer as a function of frequency in participants with hyperacusis ($p < 0.001$), irrespective of ASD status. ABR wave I amplitudes were significantly reduced at higher stimulus intensities only in participants with hyperacusis ($p=0.029$), irrespective of ASD. Wave latencies and wave III and V amplitudes did not differ based on hyperacusis or ASD status.

Conclusions: Hyperacusis is associated with subclinical peripheral auditory dysfunction in young adults with clinically normal hearing. These findings were consistent for neurotypical and autistic participants, suggesting that hyperacusis may be a transdiagnostic condition with shared mechanisms across multiple clinical populations. Subsequent analyses will identify central and autonomic markers of hyperacusis in individuals with and without ASD.

SA23. A Multimodal, Biomarker-Based Approach to Objectively Classify Tinnitus in Mice

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Background: Investigating tinnitus mechanisms in animal models requires an objective method to identify tinnitus while disentangling mechanisms underlying associated hearing loss. Based on preliminary findings, GABAA receptor binding potential may be elevated in tinnitus patients. We aim to assess viability of this biomarker for objectively classifying tinnitus in animals and compare it to existing methods for tinnitus classification.

Methods: Awake mice were bilaterally exposed to 10-16 kHz noise at 110, 115, or 120 dB for 1h, followed by a 12-week assessment. Tinnitus classification was evaluated using gap prepulse inhibition of the acoustic startle reflex (GPIAS), auditory brainstem response (ABR) wave V (inferior colliculus) to I (cochlear nerve) amplitude ratios to assess central gain, and PET imaging with [¹¹C]flumazenil to assess GABAA receptor binding. Inner ears and brains were collected for subsequent molecular analyses.

Results: At 12.5 kHz, the 110, 115, 120 dB noise-exposed groups had permanent threshold shifts of 20, 38, and 63 dB, respectively. GPIAS revealed behavioral evidence of tinnitus in the 115 dB group one week after noise exposure, persisting throughout the follow-up. In this group, ABR measurements also demonstrated increased central gain. In total, from the 115 dB-exposed mice, 4 out of 9 were classified as tinnitus-positive based on behavioral evidence of tinnitus via GPIAS and 3 out of 7 based on elevated ABR wave V/I ratios. Importantly, these two methods did not yield overlapping classifications.

Conclusions: This study establishes 115 dB bilateral noise exposure as an optimal stimulus for moderate chronic hearing loss. While GPIAS and ABR both detect evidence of tinnitus, their classifications differ, underscoring the need for a multimodal approach for robust and translatable tinnitus classification in preclinical research. Ongoing analysis of inner ear and brain tissues will reveal potential histological differences between classified groups. Ongoing

PET analysis and upcoming autoradiography experiments will determine whether GABAA receptor binding potential correlates with tinnitus classification based on GPIAS and/or ABR.

SA24. Triple Network Alteration in High β With Cochlear Implantation in Tinnitus: Source Level Resting State Functional Network Study

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Background: Cochlear implantation (CI) restores hearing and often alleviates subjective, non-pulsatile tinnitus. Meta-analyses shows clinically meaningful reductions in tinnitus questionnaire scores after CI despite substantial heterogeneity. However, the neural mechanisms by which reafferented input from the CI reorganizes large-scale cortical networks to alleviate tinnitus remain unclear. Guided by the framework of sensory reafferentation and the triple-network model of large-scale brain organization (salience network: SN, central executive network: CEN, and default mode network: DMN), we hypothesized that restored input from the CI (when it is turned on: CI ON) would reduce pathological engagement of attentional control networks, whereas transient removal of input (CI OFF) would increase their segregation. Thus, CI ON/OFF offers a within-subject probe of how sensory reafferentation reorganizes large-scale cortical networks at rest, particularly the interconnection between the DMN and attentional control circuits.

Objective: To test whether CI state (ON vs OFF) acutely reshapes functional network topology in tinnitus associated with sensorineural hearing loss and to localize any effects to large-scale networks.

Methods: Resting-state EEG was acquired during CI ON and CI OFF from 37 adult CI users with tinnitus (18 female; mean age 61.9 years). Mean deafness duration was 18.1 years; mean CI use 3.8 years; five were bilateral users. All completed the Tinnitus Handicap Inventory. EEG (64-channel BioSemi ActiveTwo) was preprocessed in EEGLAB/MATLAB. Cortical sources were reconstructed by minimum-norm imaging to estimate current source density. Weighted functional connectivity was quantified with the weighted phase-lag index, and graph-theoretic metrics were evaluated. Proportional thresholds (5–50% in 5% steps) were applied; paired statistics were corrected for multiple comparisons.

Results: High- β (18–30 Hz), global network strength was greater with CI OFF than CI ON across three consecutive proportional thresholds (20%, 25%, 30%), and the effect survived multiple-comparisons correction ($p = .001, .001, .002$ respectively). No other frequency band showed global differences. Nodal analysis in the high- β band showed higher local efficiency with CI OFF within CEN, dorsal attention network (DAN), and SN, indicating increased local segregation of attentional control circuits at rest. DMN topology did not differ by CI state. Effects were robust across thresholds from 5% to 50%; no consistent CI-state effects were observed outside high- β .

Conclusions: Acute deafferentation (CI OFF) selectively increases high- β segregation within executive-control and attentional networks, whereas reafferentation (CI ON) relaxes this configuration. This state-dependent pattern supports a network-specific account in which SN/CEN are preferentially engaged by salient internally generated auditory signals while the DMN remains comparatively stable. The results are consistent with CI ON/OFF modulation of cortical networks and complement meta-analytic evidence of tinnitus improvement after implantation despite interstudy heterogeneity. High- β local efficiency thus emerges as a

candidate systems-level biomarker to stratify tinnitus phenotypes and to guide adjunct neuromodulatory strategies targeting β -band control.

SA25. Neural Correlates of Decreased Sound Tolerance Disorders: Insights From Task-Based fMRI Study

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Background: Sounds that do not bother most people—a neighbor chewing, a pen clicking, or the hum of machinery—can be intolerable for individuals with decreased sound tolerance (DST) disorders. DST includes clinically distinct but often co-occurring disorders, most notably hyperacusis and misophonia. Hyperacusis involves increased sensitivity to sounds above a certain loudness threshold, causing discomfort or pain. By contrast, misophonia is characterized by intense negative emotional reactions to specific “trigger” sounds (e.g., chewing or sniffing), regardless of their volume. Despite growing clinical recognition, the neural mechanisms underlying these disorders remain poorly understood. Prior neuroimaging studies have typically examined hyperacusis and misophonia in isolation and without accounting for comorbidity. To address these gaps, the present study aims to delineate the neural correlates of hyperacusis, misophonia, and their co-occurrence using task-based fMRI with emotionally valent sounds.

Methods: Ninety-two young adults were recruited and categorized into four groups: misophonia only (MISO; $n = 29$), hyperacusis only (HY; $n = 14$), comorbid misophonia and hyperacusis (MiHy; $n = 24$), and age- and sex-matched healthy controls (CTR; $n = 25$). Participants completed a task-based fMRI experiment during which they listened to 90 emotionally valent sounds (30 unpleasant, 30 neutral, and 30 pleasant) from the International Affective Digitized Sounds-2 database. Participants rated each sound as pleasant, neutral, or unpleasant using button presses. Whole-brain and region-of-interest functional activation, as well as seed-to-voxel functional connectivity analyses, were conducted with a primary threshold of $p < 0.001$ and cluster-level family-wise error correction at $p < 0.05$.

Results: Behaviorally, the comorbid group (MiHy) rated more sounds as unpleasant than both MISO and CTR groups, suggesting heightened aversive perception. fMRI analyses revealed both distinct and overlapping neural patterns. MISO and MiHy (relative to CTR) showed increased engagement of visual processing regions and altered salience–visual network connectivity during unpleasant (versus neutral) sound processing, suggesting atypical cross-modal sensory involvement. The HY and MiHy (relative to MISO and CTR) groups exhibited reduced connectivity between salience hubs and frontal control regions, indicating impaired top-down regulation. Notably, in MISO, this connectivity was preserved for non-trigger unpleasant sounds, suggesting intact regulation in that context. The comorbid group (MiHy) combined both neural signatures: altered visual engagement and reduced regulatory control. Finally, both MISO and HY (relative to CTR) groups showed increased dlPFC–SMA connectivity, pointing to a shared mechanism of motor readiness.

Conclusions: Misophonia was linked to atypical cross-modal visual processing, hyperacusis to reduced top-down control, and comorbidity combined features of both, revealing distinct and overlapping neural correlates of DST. Future research should integrate neural and behavioral measures to refine mechanistic models and guide targeted interventions.

SA26. Prolonged Cortical Auditory Latencies are Linked to Poorer Executive Function and Visuospatial Memory Independent of Age and Peripheral Hearing in Older Adults

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Background: Central auditory timing reflects neural processing speed in aging and early cognitive decline, but its relation to traditional neurocognitive tests is unclear. We tested whether cortical auditory evoked potential (CAEP) latencies track executive function and visuospatial memory performance in adults with and without mild cognitive impairment.

Methods: In a cross-sectional study with 62 adults (age 57-85) at Dartmouth-Hitchcock Medical Center, we recorded the Acoustic Change Complex to an /u-i/ stimulus (primary site Cz) and derived N1 and P2 latencies from onset and change responses. Pure-tone thresholds and tympanometry assessed peripheral hearing status. Using principal component analysis a latency composite (LC1) was calculated that explained 64.1% of latency variance. This component was used as an predictor variable in the analysis to reduce dimensionality. Executive function tests were the Trail Making Test (TMT-A and B). The Rey Complex Figure Test (RCFT; copy/recall/recognition), and Brief Visuospatial Memory Test-Revised (BVM-T-R; total and T-score) were used for visuospatial memory. The associations between latency LC1 and cognitive outcomes were tested using linear regression adjusted for age, hearing thresholds (PTA), sex, and education. To assess how much the latency component added to the model, a change in R² (ΔR^2) was calculated between a model with only covariates and with covariates plus LC1. A false discovery rate (FDR) controlled multiple comparisons. Sensitivity analyses included bootstrap confidence intervals with replacement between latency LC1 and cognitive tests. A simple logistic regression explored diagnostic classification of mild cognitive impairment.

Results: Longer cortical latency was associated with worse executive performance and showed a trend toward worse visuospatial memory. Individual correlations of N1 and P2 components showed moderately-strong correlations to a variety of neurocognitive tests. For TMT-B, LC1 significantly predicted slower completion times ($\beta=0.125$, $p=0.0004$), with $\Delta R^2=0.144$ beyond covariates alone. The bootstrap 95% CI for $\beta=[0.046, 0.218]$ supported the strength of the finding. TMT-A showed a smaller effect ($\beta=0.049$, $p=0.033$; $\Delta R^2=0.034$); bootstrap 95% CI for $\beta=[0.004, 0.103]$. Associations with RCFT copy ($\beta=-0.717$, $p=0.069$) and BVM-T-R total ($\beta=-1.081$, $p=0.051$) trended in the expected direction. RCFT recall and BVM-T-R T-scores were not significant. A logistic regression using latency and covariates did not achieve useful discrimination among those adults with and without mild cognitive impairment ($p=0.079$, accuracy=66%, AUC=67).

Conclusions: Prolonged cortical auditory latencies were linked to poorer executive function and visuospatial memory, independent of age and peripheral hearing in older adults. Auditory cortical timing offers an objective and scalable neural index that could complement audiometry and speech-in-noise testing to flag cognitive vulnerability in otolaryngology and audiology settings. Future work will establish impairment cutoffs, assess longitudinal prognostic value versus PET/MRI biomarkers, and examine additional latency measures of cortical processing in background noise.

SA27. Human Auditory Cortex Encodes Multiscale Spectrotemporal Energy and Higher-Order Spectrotemporal Patterns Predictable From Deep Neural Network Models

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Background: Much of the information in natural sounds is conveyed by complex patterns of spectrotemporal modulation in a time-frequency representation of sound. Yet, how these modulations are encoded in the human auditory cortex remains poorly understood, in part due to the coarse resolution of non-invasive neuroimaging methods.

Methods: We measured human intracranial responses from 35 neurosurgical patients listening to a diverse set of natural sounds, including speech, music, animal vocalizations, and other environmental sounds (each 2 seconds). We then compared the neural prediction accuracy of three encoding models: (1) a standard spectrotemporal receptive field (STRF) model (2) a multiscale spectrotemporal energy (MSTE) model (3) a deep neural network (DNN) model trained to recognize speech, music, and environmental sound categories from a spectrotemporal representation of sound.

Results: We show that multiscale spectrotemporal energy predicts spatiotemporally precise human intracranial responses to natural sounds with much higher accuracy than standard spectrotemporal receptive field models. The MSTE model showed higher prediction than the STRF baseline throughout primary and non-primary auditory cortex and nearly tripled the prediction accuracy of non-primary, category-selective regions. The MSTE model revealed large-scale spatial maps of modulation energy, spanning primary and non-primary auditory cortex, that helped to predict the anatomical location of speech- and music-selective regions. We also observed higher-order selectivity for speech and music structure that could not be predicted by our MSTE model but could be predicted using late layers of our DNN model.

Conclusions: Our findings reveal how the spectrotemporal structure of natural sounds is encoded, transformed, and organized across the human auditory cortex

SA28. Whole-Brain Connectome Changes Following Hearing Recovery

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Background: Hearing loss induces widespread neuroplastic changes, not only within auditory cortical regions but also across non-auditory networks. These alterations include cross-modal plasticity and reorganization of higher-order cognitive areas. Although auditory rehabilitation is known to partially reverse such changes, few studies have investigated the longitudinal structural connectome alterations following hearing recovery. Understanding these changes may provide valuable insights for optimizing postoperative treatment and rehabilitation strategies.

Methods: Seven patients with congenital hearing loss were recruited under the approval of the Keio University School of Medicine ethics committee. Diffusion-weighted imaging

(DWI) was acquired at three time points: preoperatively, 3 months postoperatively, and 6–12 months postoperatively. Data were analyzed using MRtrix3 software, applying constrained spherical deconvolution (CSD) tractography to overcome limitations of diffusion tensor models. Whole-brain regions of interest (ROI) were defined in two parcellation schemes (84 and 102 ROIs), and graph-theoretical network analyses were performed. Special focus was placed on Brodmann areas 7 (superior parietal lobe), 17 (primary visual cortex), 24 (anterior cingulate cortex), and 41 (primary auditory cortex). Connectivity changes were evaluated intra-hemispherically and inter-hemispherically across the three stages.

Results: Six patients were included in the final analysis (one excluded due to incomplete follow-up). Whole-brain analysis revealed a general increase in structural connectivity over time. Detailed data will be presented on site.

Conclusions: Our findings indicate that hearing recovery surgery promotes progressive neuroplastic reorganization within the auditory connectome. Detailed data will be presented on site.

SA29. Automated Word Timing From Continuous Speech Audio for Neural Signal Analysis

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Background: An important part of speech processing is the brain's identification and interpretation of words. The brain generates neural responses synchronously with (time-locked to) the presentation times of each word, and components of these responses are implicated in higher-level contextual processing, such as surprisal or prediction. To accurately derive such neural responses to words, the exact timing of the words in a speech stream must be known in order to align them with the neural responses they elicit. However, extant word alignment tools produce coarse timings with systematic biases that make them unsuitable for this task without manual intervention. We present and validate a new method to derive accurate, unbiased word timings from an ensemble of commonly used word aligners.

Methods: An initial pass at word timings was generated by several speech-to-text alignment tools. Incorrect timings produced by these models were corrected by hand – roughly 50% of all generated timings. The timings were then estimated from the ensemble of alignment tools using a linear regression framework. The regression enabled systematic biases of certain phoneme categories to be corrected, and optimally aligned with the hand-corrected times.

Time-locked neural responses to both model-predicted and by-hand times were estimated using the Temporal Response Function (TRF) framework.

Results: The results were model-predicted timings that closely matched those that were manually corrected. The model-predicted timings produced qualitatively similar time-locked neural responses (TRFs) to the by-hand times, but without the necessity of corrections made by hand.

Conclusions: The ability to generate precise word alignment efficiently in brain imaging studies, with minimal manual intervention, will allow researchers to more easily investigate brain activity at higher-level stages of speech processing.

SA30. Silencing Auditory Cortex After Sound Offset Impairs Tracking of a Learned Sound

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Background: How sounds acquire communicative meaning through learning is a central question in auditory neuroscience. Our previous work using an ethological sound learning paradigm, demonstrated that (1) auditory cortex (ACx) is necessary for learning sound cues predictive of a social reward (Dunlap et al, *Horm and Behav*, 2020); (2) female mice trained to use sound cues exhibit enhanced cortical firing at the communicative sound's offset (Chong et al, *J Neurosci*, 2020); and (3) auditory cortical activity is prognostically modulated by decision outcomes while performing the task (Lu et al, *bioRxiv*, 2024). We have hypothesized that a learned sound's meaning is reflected in the offset responses of auditory cortex, which enable the integration of sound sequences over time into holistic auditory objects. Here, we tested this hypothesis by optogenetically silencing ACx specifically during offset responses after learning.

Methods: Naïve female CBA/CaJ mice received bilateral injections of AAV-hSyn-eNpHR3.0-EYFP into ACx and were implanted with optic ferrules. After 3 weeks of viral expression, mice were trained to track the playback of an artificial sound to choose the T-maze arm where they would be given a pup to retrieve – a highly stereotyped and rewarding behavior normally elicited by pup ultrasonic vocalizations. We used a sequence of Gaussian noise bursts (60 ms duration, 140 ms gap), band passed between (30-50kHz). Eight mice were trained in the task (100 trials/day); one did not improve with training and was removed. Three mice have reached our learning criterion so far (binomial test, two consecutive days > 68%) within 8-20 days. Performance was then tested over four days under three trial conditions (randomly interleaved, 150 trials/day): (1) no inactivation (50% Control trials); (2) optogenetic silencing during the middle of each noise burst (~25% Middle trials, LED started 20 ms after the sound onset, lasting 20 ms and linearly decaying over 20 ms); and (3) silencing immediately after each noise offset (25% OFF trials, same LED pulse).

Results: Our preliminary results with the 12 test sessions from the three animals showed that silencing ACx in the OFF window significantly impaired task performance compared to Control trials without inactivation ($p < 0.001$, Wilcoxon signed rank test). Silencing ACx during Middle trials had no significant effect ($p = 0.380$).

Conclusions: These results suggest that ACx activity after sound offset may be crucial for retrieving newly learned sound associations and/or using them to guide decisions. Ongoing work will further test this effect to clarify the role of Off responses in auditory learning. Funding provided by NIH R01 DC008343 (RCL).

SA31. Contribution of Descending Auditory Corticofugal Projections to the Spectral and Spatial Response Properties of Neurons in the Inferior Colliculus

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Background: The descending projection from the auditory cortex to the inferior colliculus (IC) has been implicated in auditory spatial learning, the passive processing of speech-in-

noise, innate defensive fear behavior, and processing of behaviorally-salient stimuli. However, stimulation of auditory cortical inputs has both excitatory and inhibitory effects on the activity of IC neurons and some response properties, such as the spectrotemporal tuning properties of these neurons and their contrast gain control, seem to be independent of cortical inputs. Therefore, how auditory cortex influences the response properties of IC neurons is far from being understood.

Methods: We optogenetically activated corticocollicular neurons in anesthetized ferrets and recorded IC activity using Neuropixels probes to explore the effect of these descending inputs on the frequency selectivity and spatial sensitivity of IC neurons. Channelrhodopsin (ChR2) was expressed in corticocollicular neurons by injecting retrograde virus encoding Cre recombinase (Retro2 AAV.Cre.GFP) at multiple sites in the IC and a viral construct encoding ChR2 in reversed fashion under the FLEX cassette (AAV.Flex.ChR2.mCherry) at multiple locations in the primary auditory cortex (A1) 4-6 weeks before the recordings. Activation of ChR2 expressed in corticocollicular neurons was achieved by blue laser light illumination delivered by an optic fibre with an intensity of 10 mW at the tip, which was placed in the center of the middle ectosylvian gyrus, where A1 is located.

Results: Activation of corticocollicular inputs increased the magnitude of the driven responses to pure tones of most neurons recorded in the central nucleus (CNIC) and the dorsal (DCIC) and external cortex (ECIC) of the IC, while decreasing their frequency selectivity in DCIC and ECIC but not in the CNIC. However, some neurons (24%) showed the opposite effect. Activation of the corticocollicular pathway shifted the frequency tuning of IC neurons, increasing the representation of the most sensitive mid-frequency region (around 10 kHz) in the ferret audiogram. The responses of IC neurons to broadband noise presented diotically were reduced upon concomitant corticocollicular activation, whereas responses to sounds presented in virtual acoustic space became stronger and more broadly tuned, especially for the contralateral hemifield. Preliminary results with recordings under anesthesia, show reduced plasticity in the spatial response properties of IC neurons with a virtual unilateral earplug when the auditory cortex was optogenetically activated.

Conclusions: Our results demonstrate pronounced effects of cortical activation on both the spectral and spatial response properties of IC neurons, paving the way for future experiments in behaving animals to explore the role of this descending circuit in active listening.

SA32. Brain Plasticity Explains Why Deaf Adult Mice Receiving Cochlear Gene Therapy Rapidly Develop Normal Hearing

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Background: Clinical reports from otoferlin gene therapy trials suggest that the emergence of hearing defined behaviorally (e.g., pure tone detection thresholds) can outpace the degree and rate of auditory brainstem response maturation. This may reflect a progressive maturation of neural synchrony and responsiveness in the early auditory pathway. Alternatively, temporal integration and central gain at higher stages of auditory processing could amplify diminished inputs from the brainstem, producing normal cortical sound representations despite persistently reduced early auditory responses. We tested these two competing models by tracking the day-to-day maturation of sound-evoked activity at every stage of the auditory

neuroaxis in otoferlin-deficient Deaf5 mice that receive otoferlin gene therapy as young adults.

Methods: Deaf5 mice were treated at 6wks postnatal with a research grade hybrid dual-AAV encoding mouse *otof* in the left posterior semicircular canal. Day-to-day changes in sound-evoked ABRs and contralateral cortical auditory evoked potentials (CAEPs) were tracked with chronically implanted microwire arrays in awake, head-fixed mice. We also tracked day-to-day responses in hundreds of chronically tracked cortical pyramidal neuron responses via 2-photon calcium imaging in triple transgenic Deaf5 x VGlut1-cre x jRCaMP8s mice.

Results: Deaf5 mice have no ABR, CAEP, or single neuron calcium responses prior to AAV treatment. Sound-evoked CAEP responses emerged just 48 hrs after treatment and reached age-matched wild-type amplitudes and thresholds within one day (N = 4/4 treated/WT mice). In contrast, ABRs only emerged after a week, with potentials emerging in reverse chronological order, from Wave V to I. In contrast to the CAEP, early ABR wave amplitudes were persistently lower than wild-type controls throughout the first 4 weeks post-treatment, particularly when measured with standard stimulus presentation rates. We then captured the onset of adult sound processing at cellular-resolution with 2-photon calcium imaging (N = 4 treated mice, n = 3,318 neurons). Well-tuned, tonotopically organized cortical receptive fields were observed 48 hrs after treatment. The emergence of inhibitory receptive fields lagged excitatory receptive fields by several days. Cortical neural ensembles decoded pitch-shifted English phonemes at comparable accuracy to age-matched normal hearing controls < 5 days after treatment.

Conclusions: We find that sound-evoked cortical activity emerged within two days of AAV treatment, a week before early ABR waves were measurable, and was indistinguishable from age-matched controls with normal developmental hearing experience within several days. Our results suggest that unlike subcortical sound encoding, which bears indelible marks of developmental deprivation, cortical circuits for temporal integration and gain control support normal sound processing despite the absence of auditory experience throughout development.

SA33. Encoding Auditory Space in Marmoset Cortex

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Background: A century ago Jakob von Uexküll argued that every species inhabits its own Umwelt – a subjective perception of the physical world shaped by its sensory and motor capacities. For foveate primates such as the common marmoset, vision captures only the frontal space, leaving the vast rear hemisphere to be monitored acoustically. How does auditory cortex meet this ecological challenge, and how is its code influenced by visual events that compete for attention in the frontal field?

Methods: We recorded single units in the auditory cortex of awake marmosets in response to 200 ms broadband noises presented from loudspeakers spanning -180° to $+180^\circ$ in azimuth. In alternate sessions a wide field visual image ($\pm 40^\circ$ azimuth) was presented at 0° , providing a frontal visual stimulation. Eye position was continuously tracked with an infrared eye tracking system. Spatial tuning curves were analyzed with circular statistics to estimate best azimuth and tuning width.

Results: Three principles emerged. (1) Direction–sharpness coupling. Tuning sharpened progressively toward the contralateral pole, culminating in a dense cluster of narrowly tuned neurons at -90° . Fisher information analysis revealed that this non uniform distribution

maximizes directional precision at midline 0°— exactly where gaze can be rapidly deployed. (2) Vision dependent gain without remapping. The frontal wide field image multiplicatively scaled firing rates yet left best azimuth unchanged, revealing a cross modal gain field rather than a spatial realignment. (3) Rear space enhancement. Gain modulation of firing rates was strongest in the contra-rear field, suggesting that visual stimulation in front strengthens monitoring of the invisible rear hemifield.

Conclusions: These results outline a dual strategy for constructing a 360° auditory Umwelt: stable directional “anchors” anchored by contralateral sharp tuned neurons, and dynamic gain control that boosts regions left outside the visual fovea when frontal space becomes behaviorally loaded. Our findings bridge classic spatial hearing theory with Umwelt thinking and offer a neural explanation for how primates maintain situational awareness while looking forward.

SA34. Visual Stimulation Alters the Stability of Echo Perception

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Background: Echo suppression with the Precedence Effect is the brain's ability to minimize distracting reflections by localizing sounds according to the first-arriving wave (lead). This process is not purely auditory: Bishop et al. (2011) showed that visual cues coinciding with a lead increase one-event response, whereas cues with the lag increase two-event response. Whether visual capture of spatial localization occurs simultaneously at the echo threshold, and whether any bias favors the lead or lag, remains unclear. In this study, we investigated how visual stimulation influences both echo response and saccade localization to test the stability of echo perception.

Methods: Twelve normal-hearing adults participated in the experiment. Lead-lag pairs of 15 ms broadband noise bursts were presented from two loudspeakers positioned at $\pm 22^\circ$ in front. Lead-lag delays varied from 0 to 14 ms in 2 ms steps. A 50 ms LED was presented at either the lead or lag position, synchronous with the lead. After central fixation, participants made a saccade toward the perceived direction of the first sound, then pressed a button once to report hearing one event or twice for hearing two events. Eye position and button responses were continuously recorded with an infrared eye-tracking system. Echo response and eye movement were analyzed across delays and visual positions.

Results: Compared to auditory-only conditions, visual cues did not affect one-event response at short delays (0-4 ms) or two-event response at long delays (12-14 ms). At echo threshold (6-10 ms), echo responses varied widely across listeners, and no reliable group-level effect of visual cue position was found. All participants showed lead-dominant localization at 2–14 ms delays. However, visual cues produced two distinct patterns of saccade localization: eight participants exhibited visual capture toward the LED, while four showed repulsion toward the lag, creating a bimodal localization pattern and greater ambiguity between lead and lag directions.

Conclusions: Visual stimulation can flexibly modulate echo suppression - either reinforcing lead dominance or weakening it to allow lag cues to emerge. These dual effects reveal a dynamic perceptual regime in which echoes can be reinterpreted through cross-modal cues, informing models of multisensory spatial integration and echo processing.

SA35. Auditory Selective Attention is Impacted by the Temporal Coherence of a Task-Irrelevant Visual Stimulus in Ferrets

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Background: Multisensory integration is a fundamental property of mammalian sensory systems, allowing us to combine information about objects in the world across senses. Audio-visual integration is especially relevant for understanding auditory processing, as visual information impacts auditory scene analysis, multi-modal object formation and speech processing.

Methods: We trained ferrets on an auditory selective attention go/no-go task, requiring them to respond to the presence of a brief timbre deviant embedded in a target stream while ignoring those in a simultaneous non-target stream. Both streams were independently amplitude modulated artificial vowels, with the target stream cued by beginning 1s earlier than the non-target, and the non-target stream attenuated relative to the target stream by 6-8 dB, with levels determined by the ferrets' performance to avoid floor and ceiling effects. 25% of trials had no timbre deviants in either stream, requiring animals to hold for the full duration of the stimulus. Streams were 5 – 6.2s in duration, and target and non-target streams differed in both pitch and vowel identity (counterbalanced across trials). Each trial had a visual stimulus in which luminance modulation was temporally coherent with amplitude modulation in either the target, non-target or neither stream. Importantly, the auditory feature coherent with the visual stimulus (amplitude) was not informative about the feature used for the detection task (timbre). Properties of the visual stimulus were selected based on those that elicited the greatest impact on neural activity in auditory cortex recorded from ferrets presented with similar audio-visual stimuli in a passive context.

Results: Ferrets performed the task well, and response selectivity (d') was higher when the visual stimulus was coherent with the target stream compared to the non-target stream (0.94 – 1.52 vs 0.84 – 1.2, across 3 animals). The specific effects of visual coherence differed across animals, with d' changes in one animal driven by a higher hit rate in the target-coherent condition, while the two other animals additionally showed an increase in false alarms to non-target deviants when the visual stimulus was coherent with the non-target stream. Ongoing work involves recording neural activity from auditory cortex during task performance to explore how audio-visual integration and selective attention interact to shape the representation of sound in auditory cortical neurons.

Conclusions: Results are in line with previous findings in a human version of this task, suggesting that temporal coherence results in the integration of visual and auditory information to create a cross-modal object.

SA36. Enhancing Music Perception Through Auditory-Vibrotactile Training

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Background: Hearing loss can impair the ability to process complex auditory information, such as perceiving speech or music in noisy environments. Even with hearing aids or cochlear implants, limitations persist in encoding fine acoustic details. These deficits reduce

speech understanding and restrict access to music, an essential domain of cultural and emotional life. Auditory-tactile integration provides a promising strategy to address these challenges. Converging evidence indicates that vibrotactile feedback can complement auditory inputs by conveying rhythm, intensity, frequency, or other auditory information. Vibrotactile input engages the somatosensory system, and possibly the auditory system, and may offer a complementary channel for sound perception, that may support everyday listening.

Methods: Our research investigates whether vibrotactile information delivered through a novel technology, the Multichannel Vibrotactile Glove, can enhance auditory perception of short musical excerpts in noise. The glove enables high-fidelity conversion of complex acoustic signals (speech, music) into vibrotactile stimulation, either uniformly across fingers or tonotopically mapped to mimic the auditory system's frequency organization. Participants are randomly assigned to one of three groups: (1) control, auditory-only training; (2) auditory training with uniform vibrotactile feedback; or (3) auditory training with tonotopically mapped vibrotactile feedback. All groups complete a five-session protocol over two weeks, including baseline assessments, three days of training, and post-training evaluation. Auditory perception is measured with the Music-in-Noise Task (MINT), which requires participants to judge whether musical excerpts embedded in noise are the same or different upon repetition. In parallel, neural correlates of sound processing are assessed with EEG during the pre- and post-training sessions, focusing on reconstruction of the acoustic envelope of continuous music as a marker of neural encoding.

Results: Two pilot studies validated the feasibility of this approach. In the first, participants showed improved MINT performance in the auditory-tactile condition, when clean melodies were simultaneously presented through vibrotactile input. In the second, participants receiving combined auditory and vibrotactile stimulation demonstrated additional gains after repeated training, suggesting that vibrotactile feedback provides immediate benefits while also supporting learning over time. We expect EEG data to show enhanced envelope reconstruction for audio-tactile stimulation compared to auditory or tactile input alone. Together, these findings provide encouraging evidence that vibrotactile cues can enhance auditory perception and strengthen neural encoding of sound.

Conclusions: Our preliminary results highlight the potential of tactile feedback to improve auditory perception and engage mechanisms of multisensory plasticity, underscoring the value of multisensory approaches for auditory rehabilitation. If confirmed, these findings could inform the development of novel rehabilitative strategies to support individuals with hearing loss in everyday listening environments. The key take-away is that vibrotactile information delivered through wearable haptic devices has the potential to complement auditory input, promote neural plasticity, and ultimately improve communication outcomes for individuals with hearing loss.

SA37. Evaluating AMPA-Type Glutamate Receptor Saturation at Inner Hair Cell Ribbon Synapses With Glutamate Uncaging and Competitive Inhibition

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Background: AMPA-type glutamate receptors in the mammalian brain mediate fast neurotransmission and are located in the postsynaptic densities (PSDs). Across synapse types,

the degree to which AMPA receptors are saturated with glutamate during synaptic transmission has been debated. In the mammalian inner ear, glutamatergic synapses are formed between inner hair cells (IHCs) and spiral ganglion neurons (SGNs). A key feature of the presynapse (IHCs) is the presence of a 'synaptic body' or 'ribbon' that organizes synaptic vesicles, modulating rates of glutamate exocytosis. Postsynaptic terminals of SGNs are characterized by large PSDs, 5 to 10 times bigger than those found in the brain. Responses to glutamate uncaging over the whole PSD have suggested that only a fraction of receptors are activated by even the largest events of synaptic transmission (ARO2024 poster, Goutman et al.). We speculate that spontaneous neurotransmission produces an overall low saturation, and therefore low desensitization, of the AMPA receptors in this PSD.

Methods: Explants of the organ of Corti from C57BL/6 mice aged 15-18 days were used. Responses to glutamate uncaging, or glutamate release by IHC evoked by high K⁺ were recorded by patch-clamp directly on SGNs terminals. Uncaging was produced by a 405 nm laser photolyzing the caged compound MNI-glutamate. Different laser intensities were used to produce glutamate transients of varying amplitudes. Competitive antagonism by 1 mM γ -DGG of responses to glutamate uncaging (uEPSCs) or high K⁺-evoked EPSCs (vEPSCs) was used to estimate the saturation level of AMPA receptors. Delivery of drugs was implemented locally through a theta-tube pipette.

Results: The low-affinity competitive antagonist γ -DGG was used to determine the level of saturation of AMPA receptors, as the percentage of block depends on the glutamate concentration. uEPSCs evoked on SGNs with high laser intensity averaged 1092 pA in amplitude and 34% block by γ -DGG, whereas uEPSCs produced by low intensity presented an amplitude of 131 pA and 60% block, with a linear relationship within a range of intermediate laser pulses. In recordings with approximately 1000 events each, vEPSCs had average amplitudes of 229 pA and block of 59%. Analysis of γ -DGG block of the 10% largest and 10% smallest vEPSCs showed differences in percentage of block that are consistent with concentrations of glutamate that are intermediate between those of uEPSCs evoked by low and high laser intensities.

Conclusions: The effect of γ -DGG on uEPSC evoked with varying laser intensities is consistent with AMPA receptors activated by different glutamate concentrations. This is also true for vEPSCs of different sizes. The overall percentage block of these synaptic currents suggests unsaturated receptors during neurotransmission between IHCs and SGNs.

SA38. Modulation of D-Serine Sensitive NMDAR Signaling as a Therapeutic Avenue for Excitotoxic Auditory Synaptopathy

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Background: Excessive glutamate release at the inner hair cell (IHC)–spiral ganglion neuron (SGN) synapse, triggered by noise, ototoxins, or aging, drives AMPAR-mediated excitotoxic synaptopathy. This pathology, marked by terminal swelling, ribbon synapse loss, and reduced auditory nerve output despite normal thresholds, underlies noise-induced “hidden hearing loss.” In contrast, cochlear NMDARs remain largely silent under physiological conditions but can be engaged under pathological stress. Since D-Serine, synthesized by serine racemase

(SR) in cochlear cells, acts as an NMDAR co-agonist, its modulation may rebalance NMDAR and AMPAR activity during excitotoxic insult.

Methods: We examined the role of D-Serine-induced NMDAR activation in cochlear synaptopathy and repair using two models: (i) a kainate-induced synaptopathy model in neonatal (P3) mouse cochlear explants, and (ii) an impulse noise exposure model (146 dB SPL, 1 pulse/s, 700 s) in vivo. Molecular assays, ultrastructural analysis, auditory brainstem response (ABR) recordings and acoustic startle reflex tests were combined to assess D-Serine's contribution to NMDAR function and excitotoxic pathophysiology.

Results: In the presence of NMDA, D-Serine significantly reduced kainate-induced acute synapse loss in cochlear explants and protected against impulse noise-induced threshold shifts in vivo. D-Serine-treated animals exhibited preserved ABR wave I amplitudes and higher synapse counts compared to noise-exposed controls. This protective effect was abolished by co-treatment with 5,7-dichloro-4-hydroxyquinoline-2-carboxylic acid (DCKA), a selective antagonist of the NMDAR glycine/D-Serine binding site.

Conclusions: These findings identify D-Serine-mediated activation of silent cochlear NMDARs as a protective mechanism against AMPAR-driven excitotoxic synaptopathy. Targeting D-Serine signaling may therefore represent a promising therapeutic strategy for auditory disorders associated with excitotoxic damage.

SA39. Modelling Auditory Nerve Demyelination upon Induction of Schwann Cell Loss in Mature Adult and Aging DTA Mice

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Background: Demyelinating diseases of the PNS can be either inherited, such as Charcot-Marie-Tooth (CMT), or acquired from an autoimmune insult, trauma or nerve injury. In these diseases, a substantial damage to Schwann cells (SCs) lead to loss of the myelin sheath and subsequent impaired peripheral nerve conduction. Enhancing the endogenous remyelination potential of the PNS is a promising therapeutic approach for promoting functional recovery and axonal survival in these demyelinating diseases. PLP-CreERT;ROSA26-eGFP-DTA (DTA) mouse is an established model for studying the effects of the specific ablation of Plp1-expressing glial cells in the nervous system upon induction of expression of the diphtheria toxin A subunit. Previous work has shown that robust SC regeneration and axonal remyelination occur in the auditory nerve a few weeks after SC ablation in young DTA mice. The process of glial cell regeneration and ultimately remyelination in aging auditory system has not been investigated yet.

Methods: Three age groups (mature-adult; 4-month-old, middle-aged; 9-month-old and aged; 12 to 14-month-old) of PLP-CreERT;ROSA26-eGFP-DTA mice, both males and females, were treated with 4-hydroxytamoxifen (labelled as DTA mice) for three to four continuous days (Traka, 2019) to induce Cre-recombination and cell death in Plp1-expressing glial cells in the PNS. Uninduced PLP-CreERT;ROSA26-eGFP-DTA littermates were used as controls. Recovering DTA mice are collected at different time points post-induction (p.i. 1, 3 and 5 weeks). Inner ears are fixed, then spiral ganglion and auditory fibers are dissected and immunoassayed to label different types of glial cell and neuronal cell markers, node of Ranvier/paranode/juxtaparanode markers and macrophage cell markers. Proliferation in glial

cells is analyzed using 5-ethynyl-2'-deoxyuridine (EdU) cell proliferation marker injected 24 hours before collecting mice. Auditory brain stem responses (ABR) are used to assess hearing thresholds. Transmission electron microscopy is used to identify myelin and neuronal ultrastructure.

Results: In mature-adult DTA mice, SC density was 56%, 76% and 81% compared to controls at 1-, 3-, and 5-week p.i respectively, indicating a gradual recovery of SCs. SC proliferation showed 3.8-fold increase in DTA mice at 1-week p.i. compared to controls. ABR showed 10-20 dB shift at 4 and 8kHz at 5-week p.i. and 0.2-0.3 ms increase in peak I latency across most frequencies at both 3- and 5-week p.i compared to preinduction levels ($n = 6-10$ /age group, males only, Mixed-effects analysis with repeated measure and Tukey's multiple comparison tests, adjusted P Value < 0.05). TEM data showed generally intact myelin in both mature-adult and middle-aged DTA mouse groups, yet signs of active, ongoing myelin degradation and phagocytosis were noticed.

Conclusions: Robust SC regeneration occurs after cell ablation in mature-adult auditory system relying mainly on activated glial cell proliferation. Overall, despite the SC regeneration, persistent functional defects exist in auditory system of the aging DTA mice probably due to impaired PNS remyelination.

SA40. Objective Measures of Loudness in Cochlear-Implant Hearing

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Background: Objective measures provide a way to program cochlear implants (CIs) for patients, including infants, who cannot provide reliable reports. The Electrically Evoked Compound Action Potential (ECAP) can be measured quickly and does not require additional equipment, but the literature reveals only modest between-participant correlations with thresholds or most-comfortable-loudness levels (MCLs). In addition we (Garcia et al, JARO, 2021) showed that ECAPs can vary substantially across electrodes for a given participant even when all electrodes are stimulated at MCL. Experiment 1 tests two explanations for this discrepancy by measuring ECAPS in conditions that manipulate effects of neural synchrony and refractoriness. Experiment 2 combines and compares objective measures of the neural response of multiple stages of the auditory system to the same CI stimulus, to further probe this discrepancy and to obtain insights into the neural bases of loudness perception.

Methods: Many ECAP studies, including Garcia et al (2021), use the masker-probe (MP) method of artefact cancellation, whereby masker and probe pulses are presented separately and in quick succession. The resulting ECAPs depend on the masker in the combined stimulus reducing the neural response to the probe via refractory effects. Differences in refractoriness could therefore cause ECAP amplitudes to differ between equally-loud stimulation of different electrodes. Experiment 1 measured ECAPs on all electrodes for 11 users of Advanced Bionics CIs, using both the MP and the alternating-polarity (AP) artefact-cancellation paradigms, as the AP paradigm is immune to refractoriness. Both conditions used symmetric-biphasic pulses. Because of evidence that auditory-nerve (AN) response is more jittered to cathodic than to anodic stimulation, we additionally used the MP method with pseudomonophasic pulses consisting of a cathodic-leading long, low-amplitude phase followed by a short, high-amplitude anodic phase. We reasoned that the jittered response to the cathodic phase of symmetric pulses might contribute to loudness but not to ECAPs, and that this effect would be reduced by the low-amplitude cathodic phase in pseudomonophasic stimulation. Experiment 2 presents loudness-balanced ~40-pps pulse trains separately to six

electrodes spaced along the array, and simultaneously measures ECAPs, EABRs, and EASSRs using recently-developed methods and a hyper-rate EEG system (Arzounian et al, Hearing Research, 2025).

Results: Experiment 1 found no evidence that the across-electrode standard deviation (s.d.) of ECAP amplitudes differed significantly between conditions. Experiment 2 (underway) will compare the s.d. in each measure across the (equally-loud) stimulation of the 6 electrodes. To determine which measure most accurately reflects loudness these s.d. values will be compared to the effect of changing stimulus level for each measure.

Conclusions: Factors other than refractoriness or neural jitter are responsible for the discrepancy between ECAP amplitudes and loudness. Simultaneous measurements of the AN, brainstem, and thalamic/cortical response to the same CI stimulus may inform the neural stages determining loudness perception.

SA41. The UmboMic: Surgical Approach and Fixation

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Background: The cochlear implant of the future will be invisible to the naked eye. Currently, several totally implantable cochlear implants (TICIs) are in clinical trials. The core challenge remains delivering comparable audio quality to external microphones without excessive noise. An implantable microphone must have long-term robustness, simple surgery, low power consumption, and high margin of safety. Of the proposed TICIs, two use subdermal microphone and one uses middle ear microphones. The subdermal microphone is low risk, but sacrifices audio quality. Middle ear microphones would enable higher fidelity hearing. For the latter, without a well-engineered fixation system, even the best sensor is impractical.

Methods: We present a novel fixation system for our UmboMic sensor, which senses umbo motion. The design must offer adequate stiffness while permitting uncoupled motion in the key degrees of freedom to simplify surgery. We used kinematic analysis, stiffness calculations, and finite element simulation to validate performance. A 3D printed temporal bone provided preliminary sizing and positioning. Surgeon feedback and temporal bone experiments refined the geometry and size of the final design. The fixation system reaches through the facial recess and facilitates contact of the UmboMic to the umbo.

Results: A stainless-steel prototype was fabricated via wire EDM, waterjet, and machining. The prototype was tested in temporal bones to verify size, placement, and positioning. A bone plate is used to anchor to the mastoid cortex and sensors are glued to the distal end of an extensible arm for positioning. The low profile design provides visual access for the surgeon. Simulations show a fixation-to-sensor stiffness ratio ≥ 10 . The components of the fixation system can be locked in place using UV-curing glue. Techniques with applications to surgery such as the use of a viscous adhesive to form a foundation upon which to rest the fixation system in the mastoid bowl and an audio feedback mechanism for determining coupling to the umbo were explored.

Conclusions: We describe the design methodology, analysis, and initial validation of a fixation system. We find the stiffness ratio must be satisfied for good microphone performance. Thus, implant-grade stainless steel is preferred and the mastoid cortex is a

suitable anchor. The minimal design uses locking revolute and prismatic joints to achieve the desired motion and stiffness with flexural mechanisms as a possible enhancement. Further improvements pertain to the locking mechanism and custom tooling for facilitating surgery, eventually leading to experiments in cadaveric heads of the full implantable microphone (sensor, amplifier, fixation system) to evaluate system performance. This is a critical and anticipated step in the development of an implantable middle ear microphone for use with future TICIs.

SA42. Fiber-Specific Modeling of Electrically Stimulated Auditory Nerves

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Background: A quantitative description of the functioning of electrically stimulated nerve fibers, as provided by a computational model, is of central importance to better leverage the potential of neuroprostheses, such as cochlear implants. However, there is virtually no openly available data of electrically stimulated auditory nerve (AN) fiber responses. Publications only offer graphic images of selective example neurons or in the form of population averages, posing severe limitations for follow-up studies, especially for modeling studies.

The goal of the present study is to demonstrate the added value of having access to a rich data set by fitting parameters of a computational model to individual fibers. As an exemplary application we compare chronic with acutely deafened fibers both with and without the model.

Methods: Spike times and voltage traces from 188 electrically stimulated auditory nerve fibers from guinea pigs, which were originally recorded by Heffer et al. (Journal of Neurophysiology 104, 2010), have been made publicly available (Heffer et al. Zenodo, 2025). 118 of these recordings were usable for this work – 74 of these fibers were from acutely deafened animals and 44 from deafening five weeks before the recordings. The stimuli were 100-ms long constant amplitude biphasic pulse trains, with multiple amplitudes and pulse rates.

We optimized the parameters of a leaky-integrator-based model of an electrically stimulated auditory nerve fiber (Felsheim and Dietz, Trends in Hearing, 2024) for the 118 fibers, to reproduce the response patterns as closely as possible. We then used the 118 fiber-specific models to extract established measures, such as refractory period or facilitation, and compared them to measures obtained directly from the recorded spike times.

Results: The response patterns of individual nerve fibers varied widely, generally in line with previous reports. When looking at the differences between the acutely deafened and the chronically deaf group, we saw that the longer deafened nerve fibers responded on average more than the acutely deafened ones. This was reflected in the model parameters, which showed a shorter refractory period for the chronically deaf fibers. For high-amplitude 1000 pps pulse trains, we found a significantly higher vector strength in the chronically deaf group and, correspondingly, a lower jitter was estimated using the model.

Conclusions: The model-based analysis of this large set of electrically stimulated auditory nerve fibers allows to analyze and explain fiber-specific response patterns as well as group differences between fibers from acute versus chronically deaf guinea pigs. In this way, more information can be extracted from existing data as with conventional analysis methods.

SA43. Effects of Phonological Working Memory and Speech Recognition in Quiet as Predictors of Speech-In-Speech Recognition in Adolescents and Young Adults With Early Cochlear Implantation

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Background: Auditory neurocognitive models suggest that degraded auditory input affects the development of mental phonological representations, which are critical for phonological working memory -- a subcomponent of working memory responsible for the temporary storage and processing of information that specifically handles speech-related information. While children with cochlear implants show deficits in phonological working memory, it remains unclear whether these deficits are related to speech recognition under challenging listening conditions, as is the case for children with normal hearing. The aim of this study was to investigate the effects of phonological working memory and speech recognition in quiet on speech-in-speech recognition in adolescents and young adults with early cochlear implantation.

Methods: Thirty-four adolescents with bilateral cochlear implants participated (mean age = 16.5 years; range: 12.8 – 21.7 years). The mean age at first implantation was 1.3 years (range: 0.6 – 2.2 years). The participants completed three tasks: recognition of speech-in-quiet, recognition of speech-in-speech and repetition of nonwords. Speech-in-quiet (SIQ) was measured at 65 dB SPL in sound-field at frontal incidence using a Swedish clinical speech audiometry test, consisting of phonemically balanced lists of 50 monosyllabic words. The outcome was the proportion of correctly identified words. Speech recognition threshold (SRT) in competing speech was assessed using an adaptive psychoacoustic procedure. The target speech consisted of Swedish Hagerman sentences and was presented from a frontal speaker, whereas the competing speech – set at a fixed level of 63 dB SPL -- was spatially symmetrically separated from the target. The threshold was set to 40% correct speech recognition. Phonological working memory was assessed using the Repetition of Nonwords (RON) task from a computer-based test battery. Participants repeated nonwords of varying complexity presented as auditory-only stimuli through loudspeakers. Performance was scored using the percentage of correctly repeated consonants. A multiple linear regression analysis was performed with SRT as dependent variable and SIQ and RON as covariates.

Results: Multiple linear regression analysis revealed that the combination of phonological working memory and speech-in-quiet scores explained 47 % ($p < 0.001$) of the variability in speech-in-speech recognition. Phonological working memory explained 28 % ($SRT = 4.5 - 15.0 \times RON$, $p = 0.001$) and speech-in-quiet 39 % ($SRT = 11.9 - 0.2 \times SIQ$, $p < 0.001$) of the variability, respectively.

Conclusions: For adolescents with early implantation, phonological working memory is a factor in the ability to recognize speech masked by speech. By integrating cognitive and audiological factors into a unified model, the present findings contribute to the understanding of how speech recognition under adverse listening conditions may be improved. Further research on this association is warranted, as supporting phonological working memory in children with CI may benefit their speech recognition in challenging environments.

SA44. Electric Acoustic Interaction Across Auditory Pathway in Cochlear Implant Users With Ipsilateral Acoustic Hearing

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Background: Cochlear implant (CI) users with electric acoustic stimulation (EAS) combine electric stimulation (ES) through the CI and acoustic stimulation (AS) through the ipsilateral residual hearing. EAS typically improves speech perception, suggesting interaction between the two modalities. Psychoacoustic studies using acoustic masking of electric stimuli have provided evidence for such interactions, showing stronger masking with greater residual hearing. Moreover, acoustic masking has been observed at the base of the cochlea. The underlying mechanisms of this interaction remain insufficiently understood. Gaining deeper insight into the fundamental neural processes of electric-acoustic interaction through masking studies could facilitate the development of less invasive extracochlear implants and objective diagnostic tools for assessing low-frequency hearing. Such advances would be particularly valuable for newborn hearing screening, where current methods for accurately evaluating low-frequency residual hearing remain limited.

Methods: Eight EAS CI users with partial insertion participated in the current study. Three electrodes were tested: most apical active (A_El), most basal active (B_El) and a deactivated extracochlear electrode located at the round window niche (RW_El). Auditory brainstem responses (ABRs), middle-latency responses (MLRs) and cortical auditory evoked potentials (CAEPs) were measured to the electric pulses presented at the most comfortable level (MCL) across three electrodes. The same recordings were then obtained in the presence of a continuous acoustic noise at MCL, to assess electric-acoustic interactions. Evoked responses were measured using conventional averaging paradigms. In addition, a novel deconvolution method capable of capturing evoked responses across the entire auditory pathway within a single recording was validated in CI users for the first time.

Results: Deconvolution proved feasible for assessing evoked responses in CI users. ABRs, MLRs, and CAEPs were consistently measurable at A_El, while responses at B_El were observed only in a subset of participants. At RW_El, evoked responses were measurable in some participants, with MLRs being the most prominent. When continuous acoustic masking noise was introduced, preliminary analyses revealed the strongest suppression of evoked responses in the cortical region.

Conclusions: It was possible to measure evoked responses at RW_El in some participants demonstrating the potential of extracochlear stimulation. While reliable responses to electric stimulation were obtained at the A_El in all participants, responses at the B_El were less consistently observed. Analysis of acoustic masking on electric stimulation revealed that the most prominent suppression of neural responses occurs at cortical level. Moreover, these preliminary findings demonstrate the feasibility of using the deconvolution method to record evoked responses and analysis the interactions between acoustic and electric stimulation in CI users.

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SA45. Attentional Processing in CI-Users and Normal-Hearing Listeners Signified by Alpha Oscillatory Activity

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Background: Cochlear implants (CIs) allow hearing-impaired individuals to gain access to speech, but the outcome variability remains high. The auditory input of a CI through electrical stimulation is in many respects different than acoustic stimulation of the normal hearing, yet the effect of this difference on cognitive processing of auditory input is not fully understood. Hearing with a CI requires more cognitive resources, involves more listening effort and requires more attentional resources. The objective in this study was to identify the oscillatory signature of attentional processing in CI users and to compare them with normal-hearing (NH) listeners.

Methods: Electroencephalographic (EEG) data (128 channels) were recorded from postlingually deafened CI users (n = 37) and NH controls (n = 37) during performance of a three-stimulus auditory oddball experiment. The paradigm consisted of frequent standard tones (Standard), infrequent deviant tones (Target) and infrequent unique complex sounds (Novel), all lasting for 400 ms. The participants performed the experiment once in an active and once in a passive condition, where in the active condition, they were instructed to respond via button press to the Target. The analysis of the EEG data included a time-frequency analysis, a reconstruction of the neural sources via beamforming and the estimation of neural activity in the transverse temporal gyrus (TTG), dorsolateral prefrontal cortex (DLPFC) and inferior parietal lobules (IPL).

Results: The most prominent oscillatory activities were found when involving attention towards the auditory stimulus (active condition, particularly in response to the Target). Attention-related oscillations were analyzed by subtracting the response to the Standard stimulus from the response to the Target stimulus. These included delta-theta synchronization, alpha desynchronization and beta desynchronization. The alpha desynchronization, observed in previous EEG studies in attention-related tasks, was prominent in both groups. Interestingly, CI users exhibited additional alpha signature also during Novel processing, suggesting that CI users elicit additional attention to the task-irrelevant stimuli. Furthermore, during Novel processing, the source reconstruction revealed an additional alpha source in CI users in the left parietal cortex. Combined with the distinct local neural activities in TTG, DLPFC and IPL, these data suggest that CI users recruit a different attentional network than NH listeners.

Conclusions: The present study revealed oscillatory markers of auditory attentional processing in CI users. CI users direct more attention towards task-irrelevant stimuli and utilize different attentional networks compared to NH listeners. In summary, the results in this study align with previous literature identifying alpha desynchronization as a reliable objective marker of attentional processing.

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SA46. AC102 Hydrogel Combined With a Dexamethasone-Eluting Cochlear Implant Electrode Protects Residual Hearing and Reduces Electrode Impedances

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Background: Cochlear implantation (CI) remains the most effective and widely accessible therapeutic intervention for individuals with severe-to-profound sensorineural hearing loss. Despite its well-established clinical benefits, CI can be associated with several limitations, such as electrode insertion trauma resulting in damage to the sensory and neural cells of the inner ear, as well as foreign body reactions leading to fibrotic tissue formation around the electrode carrier. The aim of this project was to evaluate the therapeutic potential of the promising compound, AC102, in combination with dexamethasone (DEX)-eluting CI-electrodes.

Methods: In Mongolian gerbils, AC102 formulated in a thermoreversible hydrogel was applied intratympanically either 1 or 24 hours prior to CI. Alternatively, control (CTRL)-hydrogel was injected 24 hours before intervention. Subsequently, either DEX-loaded or unloaded CI-electrodes were implanted via the round window. Thus, 5 different study groups (CTRL-/24h-AC102/1h-AC102 hydrogel and unloaded-electrode, CTRL-/24h-AC102 hydrogel and DEX-electrode; n = 10-16 per group) were followed up to 90 days with regular audiometric testing including cochlear compound action potential (CAP), and CI-electrode impedance measurements. After the study endpoint, animals were euthanized and cochleae harvested for histological analysis, including assessment of spiral ganglion neuron density and cochlear fibrosis formation, as well as quantification of hair cell and synapse survival.

Results: At the 90-day follow-up, AC102 treated animals had significantly lower CAP-threshold shifts in low frequencies (1 and 2 kHz) compared to untreated animals. This protective effect was less pronounced and insignificant in frequencies corresponding to the anatomical cochlear region surrounding the electrode tip (4 and 8 kHz). In high frequencies (16 and 32 kHz), AC102 treatment again led to lower CAP threshold shifts. Notably, CAP thresholds in these frequencies were higher in animals implanted with a DEX-electrode. Furthermore, impedances were significantly lower in animals implanted with DEX-eluting electrodes compared to animals implanted with unloaded-electrodes. Histological analysis is ongoing and will provide further insights into the cellular and structural mechanisms underlying the effects AC102 and DEX treatment.

Conclusions: This study confirms the otoprotective properties of AC102 in CI. Furthermore, it builds upon previous findings by demonstrating a synergistic effect when combined with DEX-eluting electrodes, evident in both the preservation of residual hearing and the attenuation of cochlear fibrosis. Notably, the timing of AC102 administration did not significantly influence outcomes, a factor that could support the development of flexible pre-treatment CI protocols in a clinical setting. Elevated CAP-threshold shifts at high frequencies following implantation of DEX-eluting electrodes may be attributed due to an increased rigidity of the electrode carrier, an effect likely to be less pronounced in the larger, human cochlea. Altogether, these findings underscore the promising therapeutic potential of AC102 in CI – particularly in combination with a DEX-eluting CI-electrode.

SA47. A Single-Center Study of Pupillary Dilation During Post-Auricular Vagal Nerve Stimulation in Cochlear Implantation

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Background: Rehabilitation after cochlear implantation (CI) may require neuroplasticity as patients adapt to novel electrical sound signals.¹ Vagus nerve stimulation (VNS) has been shown to enhance neurocognitive function and knowledge acquisition, with recent studies showing that concurrent auditory input and VNS drive auditory cortex neuromodulation.² Animal studies have also shown VNS to improve performance on auditory learning tasks.³ VNS may therefore have applications in post-CI rehabilitation. As Arnold's nerve, a vagus nerve branch, can be visualized during the post-auricular CI approach, this study examined whether intraoperative stimulation of Arnold's nerve affects vagal activity, indexed by pupil dilation.

Methods: This IRB-approved, prospective study included 18 CI patients. An anesthesia protocol was developed to minimize confounding effects on vagal tone. A standard post-auricular CI incision was made, followed by a modified PALVA flap to preserve Arnold's nerve branches. Stimulation occurred prior to mastoidectomy completion. Baseline pupil size was obtained with the Neurolight pupillometer. Negative-leading biphasic stimulation (via the Medtronic NIM-Response 3 system) was applied at 0.5, 1.0, and 1.5 mA across three external auditory canal sites. Stimulation was applied for 10–30 seconds with continuous pupillometry measurements. Patients were classified as “responders” or “non-responders” using a threshold of $\geq 5\%$ average change in pupil diameter. We also assessed whether current amplitude or location of stimulation affected change in pupil size among responders.

Results: Of the 18 patients included in this study, pupillometry data from 14 patients were available for analysis. Among these 14 patients, 10 (71%) were classified as responders with a mean percent change in pupil size of 9.30% (SD: 8.0%). On linear regression, the percent change in pupil size was not significantly associated with current amplitude ($p > 0.05$). All sites of stimulation resulted in similar pupil dilation ($p > 0.05$). Only one patient exhibited a significant within-subject effect of location on change in pupil diameter ($p = 0.013$). No adverse events were noted during stimulation, including facial nerve stimulation, hemodynamic instability, or laryngeal stimulation.

Conclusions: In this preliminary study, intraoperative Arnold's nerve stimulation produced significant pupillary dilation in most patients. Pupil change was not significantly influenced by current amplitude or site of stimulation. If Arnold's nerve stimulation can safely and reliably generate VNS, it may offer an accessible post-auricular target for implantable VNS devices to enhance learning during post-CI aural rehabilitation.

SA48. Developing Optimization Strategies to Compensate for Losses Due to Auditory Nerve Synaptopathy

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Background: Difficulty understanding speech in noise is the most common complaint of patients with sensorineural hearing loss. Cochlear neural degeneration (CND)—the loss of peripheral auditory nerve fibers (ANF)—has been hypothesized as a major contributor to

these deficits, yet it remains sub clinical in audiometry measurements. In the absence of noninvasive methods to probe for neural deficits, current hearing-aid (HA) fitting algorithms, such as NAL/NL2, which are based solely on audiometric thresholds, ignore the neural component of hearing loss. The first question we address in this study is how to effectively design speech stimuli that can probe deficits resulting from CND. The second question that we address in this study is how to explore the Hearing Aids parameter space to specifically address neural deficits.

Methods: We used a phenomenological model of auditory periphery (Bruce et. al 2018), to simulate the auditory nerve fiber responses to input sound stimulus. (1) To address the 1st question i.e. the effectiveness of speech materials to detect CND, we developed a Mutual information based framework that uses loss of information between inner hair cells receptor potential and ANFs to quantify the loss of encoding and reduction in information carrying capacity of ANF due to CND. (2) For the second question i.e. Hearing aids fitting, we developed a Simulation-Based Inference (SBI) pipeline to optimize HA level compression algorithms with a focus on compensating for CND. We simulated speech processing under varying degrees of sensory loss and CND. The HA parameter space, including compression threshold, slope, and attack/release times, were systematically explored within the SBI framework to identify parameter sets that minimize neural deficits. Our approach aims to identify alternative parameter combinations that could, in theory, provide better neural compensation and improve speech-in-noise performance.

Results: (1) Our mutual information framework suggests that time-compressed speech (40% and 65% dilatation) produces the greatest sensitivity to CND-related information loss, making it an effective speech probe for evaluating neural deficits. (2) Preliminary results indicate that hearing aid parameters can be explored using SBI to address CND deficits as a framework to reveal parameter-space not captured by the standard NAL/NL2 prescription, particularly for challenging listening conditions.

Conclusions: This project establishes an SBI-based approach to systematically evaluate and optimize HA parameter settings in the context of CND. By highlighting where current gain prescriptions fail, we will re-evaluate compensation strategies to better address the hidden neural deficits underlying speech-in-noise difficulties. Moreover, by exploring the larger parameter space of HA algorithms, this work could uncover fitting strategies that provide more effective compensation for CND-related deficits. [Work supported by MEE SharkTank award and grant R01 DC07910 from the NIDCD of NIH.]

SA49. Electrical and Biological Investigation of Electrospun PvdF-TrFE Fiber Mats to Reduce Fibroblast Growth After Cochlear Implantation

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Background: Cochlear implants (CI) are implanted to restore hearing in patients with severe to profound sensorineural hearing loss. Due to the foreign body response and insertion trauma, connective tissue is formed around the electrode array, which electrically insulates the implant, hampering electrical stimulation of the auditory nerve. Modification of the electrode surfaces might be a way to reduce the adhesion of connective tissue. In the current study, fiber mats consisting of the hydrophobic material poly(vinylidene fluoride-trifluoroethylene) (PVDF-TrFE) were investigated in vitro regarding electric conductivity, biocompatibility, and growth of fibroblasts on their surfaces.

Methods: Fiber mats of three different thicknesses (15, 30 and 45 min spinning time) were cut into 2x6 cm rectangular samples. Using an in-house manufactured chamber with four measuring cells, the increase of impedance and the wetting process over time was measured on conventional SEM holders. In a second step, fibers were spun directly on platinum electrode contacts of a similar size as in CI electrode arrays, which were embedded in silicone. In addition, biocompatibility tests were performed as eluate tests on primary spiral ganglion cells and NIH/3T3 fibroblasts to investigate cytotoxicity. Furthermore, the growth of eGFP NIH/3T3 fibroblasts was examined on Ø 6 mm circular samples for seven days by means of confocal laser scanning microscopy and compared to the growth on cell culture material.

Results: The increase in impedance was dependent on the thickness of the fiber mats (thinnest: $1169.63 \pm 248.32 \Omega$, thickest: $3209.64 \pm 556.72 \Omega$). Immersion in physiologic saline decreases impedance in a time dependent manner. Wetting of the mats can be accelerated by the additional use of EtOH. The only constraint in biocompatibility was found with 100% extraction solution ($66 \pm 7 \%$ cell viability for 4h drying time, $57 \pm 10 \%$ for 24h drying time). The growth of fi-broblasts is reduced on the electrospun fiber mats compared to the surrounding cell culture material (fiber mat 70.8 ± 10.9 average fluorescence intensity, $82 \pm 2.9 \%$ cell covering after 7 days), (surroundings 79.5 ± 5.7 average fluorescence intensity, $92.2 \pm 0.7 \%$ cell covering after 7 days).

Conclusions: A thin film of electrospun PVDF-TrFE seems to have the potential to reduce fibroblast growth on stimulating contacts of CI electrodes without increasing the electrode impedance too much. An appropriate strategy for wetting the mesh appears mandatory.

SA50. Comparing Psychophysical Tuning Curves (PTCs) and Spread of Excitation (SOEs) in Children and Adults With Cochlear Implants

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Background: Cochlear implants (CIs) can restore a sense of sound to people with severe hearing loss. However, while CIs are generally effective at providing speech understanding in quiet, noisy situations can still be a challenge. One possible reason is poor frequency selectivity, in which certain frequencies are more easily masked by sounds in nearby frequencies. The exact cause of this is not known, however one strong candidate is channel interaction, in which neighboring electrode channels stimulate overlapping regions of auditory nerve fibers. It has been shown that pediatric CI users perform better in vowel identification in noise than adult CI users (Arjmandi, 2025), signifying improved frequency selectivity. One reason could be that the central processing of pediatric CI users has better adapted to the CI and is less dependent on the periphery. In this pilot study, we test the hypothesis that pediatric CI users will have similar measures of channel interaction (spread of excitation or SOEs) to adult CI users but better behavioral measures of frequency selectivity (psychophysical tuning curves or PTCs) compared to adults.

Methods: Five pediatric CI users (ages 11–17, M = 14 years) with Advanced Bionics processors were compared with nine previously tested adults (ages 23–75, M = 52 years). Pediatric users were tested on electrode 8, adults on two electrodes with high and low thresholds. Three-point PTCs were obtained by measuring probe thresholds on electrode 8 in a 25 dB SPL-equivalent on-frequency masker, then raising the masker 12 dB and sweeping from basal and apical sides until detection failed. The probe was 20 ms, the masker 200 ms

with a 5 ms gap. Basal and apical slopes were then calculated. SOEs were measured with panoramic ECAPs (biphasic, cathodic-leading pulses) and fit with Gaussian curves to derive slopes. Linear regression tested whether age group predicted SOE or PTC slopes.

Results: PTC slopes ranged from 3.18–17.75 dB/electrode ($M = 8.08$) in pediatric users and 1.68–23.37 dB/electrode ($M = 8.75$) in adults. SOE slopes ranged from 0.06–0.23 ($M = 0.11$) for pediatric users and 0.004–0.25 ($M = 0.11$) for adults in proportion of max amplitude/electrode. Age group did not show a significant effect on PTC slopes ($p = 0.47$) or SOE slopes ($p = 0.94$).

Conclusions: We hypothesized that age group would not predict differences in SOE slopes but would predict sharper PTCs for the pediatric group. According to the present data, age group did not predict PTCs. Alternative measures may better account for differences in vowel performance for pediatric CI users, such as years of listening experience.

SA51. Evaluating Stimulation Strategies for Selective Activation of Auditory Nerve Fibers in a Guinea Pig Model

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Background: Selective activation of distinct neural populations is essential for high-resolution neural prosthetic systems. In cochlear implants (CIs), current spread through cochlear fluids and current shunting along the cochlear bony anatomy limit spatial precision. In contrast, the auditory nerve implant (ANI) interfaces directly with the central axons of spiral ganglion neurons (SGNs), enabling more localized depolarization and hyperpolarization of relevant neural populations. This direct interface provides a unique opportunity to enhance spatial and temporal selectivity by optimizing stimulation waveform shape and timing, an approach shown to be effective in other neuroprosthetic systems, such as motor, somatosensory, and retinal implants. In this study, we systematically evaluate strategies for improving selective activation of auditory nerve fibers by varying stimulation configurations, pulse waveform parameters, and interleaved pulse timing. We assess how these factors influence spatial activation patterns and temporal response profiles through electrophysiological recordings in the auditory midbrain.

Methods: A 2-shank, 32-site electrode array (NeuroNexus) was positioned along the tonotopic axis of the central nucleus of the inferior colliculus (ICC) in ketamine-anesthetized guinea pigs to record multi-unit activity. Acoustic responses to pure tones (1–40 kHz) across eight intensity levels (0–70 dB SPL) were recorded to validate electrode placement and generate a frequency response map for reference during electrical stimulation. A transcochlear surgical approach was used to expose the modiolar portion of the auditory nerve. A 16-site single-shank edge electrode array (NeuroNexus) was inserted into the auditory nerve bundle for electrical stimulation. Charge-balanced biphasic pulses with a 40 μ s inter-phase gap were delivered using either cathodic- or anodic-leading waveforms. Pulse durations ranged from 40 to 120 μ s. Return electrodes were configured for either monopolar or bipolar stimulation. Additionally, interleaved stimulation across neighboring sites was tested to assess spatial interaction effects.

Results: On average, bipolar stimulation resulted in higher activation thresholds but more spatially confined responses in the ICC compared to monopolar stimulation. Thresholds decreased with increasing pulse width; however, spatial variability in threshold across the 16 stimulation sites increased with shorter pulse durations. This variability was more

pronounced in the bipolar configuration. No consistent threshold difference was observed between cathodic- and anodic-leading pulses within our limited sample. Interleaved stimulation across neighboring electrodes reduced cross-channel interference and preserved spatial selectivity.

Conclusions: Our results show that stimulation configuration (e.g., monopolar vs. bipolar), waveform characteristics (e.g., pulse width), and temporal strategies (e.g., interleaving) have a significant impact on activation thresholds, dynamic range, and spatial activation patterns in the auditory midbrain. These effects are likely driven by underlying physiological factors such as electrode-to-fiber distance, axon diameter, and the distribution of the electric field. Understanding how these neural response differences relate to perceptual outcomes, such as spectral resolution and loudness growth, is essential for guiding the development of next-generation auditory prostheses.

SA52. Development and Translation of a Novel Auditory Nerve Implant to Restore Hearing in Deaf Patients

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Background: The cochlear implant is one of the most successful neural prostheses, with more than one million devices implanted into patients worldwide. However, advancing the performance of cochlear implants by better stimulating the auditory nerve has plateaued, largely due to the difficulty in directly stimulating the auditory nerve through the bony wall of the cochlea. This has limited progress in improving outcomes for real-world tasks such as understanding speech in noise and for music appreciation. Our project has developed a novel auditory nerve implant (ANI) that combines a Blackrock Neurotech ANI electrode array coupled to a MED-EL Synchrony 2 cochlear implant stimulator. The goal is to evaluate the benefits of more direct and focal microstimulation of the auditory nerve compared to a cochlear implant, which will be assessed through perceptual testing in an upcoming pilot clinical study. Extensive technology development, along with multiple pre-clinical and intraoperative clinical evaluations have been performed to pave the way for the pilot study of the ANI device, which is expected to initiate in Fall 2025.

Methods: Our pre-clinical studies included over 40 human cadaver head experiments to develop the proper surgical approach for the ANI. Additionally, a chronic cat ANI study was successfully performed with 3-month endpoints after device implantation. More recently, a first-in-human intraoperative study with nine volunteers was performed under Ethics Committee approval at the Hannover Medical School in Hannover, Germany. The electrical stimulation regimen for both the cat and intraoperative studies was evaluated through

electrically evoked auditory brainstem responses (eABRs) and electrode impedance measurements. Intraoperative experiments were performed with volunteers receiving tumor resection surgeries by a translabyrinthine approach, which is the same surgical approach planned for the upcoming ANI clinical study (future surgical approaches that do not compromise the labyrinth are also being investigated for broader patient adoption). The surgical approach, device handling, and electrode placement, as well as eABRs and electrode impedances were evaluated in the intraoperative study using a wired version of the ANI system.

Results: We found that the ANI could be safely and successfully positioned into the auditory nerve while exhibiting low stimulation thresholds between 20 and 40 μA , which are an order of magnitude lower than that observed with cochlear implant stimulation. eABR measurements showed robust activation of downstream auditory brainstem structures. Our chronic experiments in the cat model supported the sustained health of the nerve and performance of the device over clinically relevant periods.

Conclusions: Overall, our results have justified our upcoming ANI pilot clinical study, which has obtained Ethics Committee approval at the Hannover Medical School. In that study, we aim to collect first-in-human data showcasing surgical implantation of the ANI into the auditory nerve, stability and safety of the implant, and preliminary perceptual outcomes of ANI stimulation.

SA53. A Deep Learning Framework for Understanding Cochlear Implants

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Background: Cochlear implants (CIs) aim to replace dysfunctional cochlea in people with severe hearing loss by electrically stimulating the auditory nerve. Although highly successful, CIs fail to restore normal perception in many listening situations. Performance outcomes may be limited by stimulation strategies, neural degeneration, or suboptimal decoding by the brain. We propose a deep learning framework to evaluate these issues by estimating best-case outcomes with task-optimized decoders operating on simulated auditory nerve input from CI stimulation.

Methods: We optimized deep artificial neural networks to recognize words and localize sounds using simulated CI-stimulated auditory nerve representations, on the assumption that the performance of such models should approach the best that is possible given the simulated CI. Once trained we compared the models to humans as well as to models optimized for normal hearing input (all tested on the same tasks). To investigate outcomes for different device manufacturers (Cochlear, MedEl, Advanced Bionics), we separately trained models on simulated nerve activity evoked by their respective sound-coding strategy. To gain insight into other factors that might limit outcomes, we also manipulated the dynamic range and spatial spread of current, the extent of simulated neuronal loss, and the number of neural network stages optimized for CI input (simulating limits on plasticity).

Results: Best-case CI models exhibited impaired speech recognition and sound localization relative to the normal hearing model. Human CI users tested on the same task exhibited a range of behavior, with the best-performing users approaching the behavior of the best-case models. Performance was similar across the different sound coding strategies. Model performance was influenced to varying degrees by the dynamic range and spread of current,

neural survival, and the extent of neural network optimization, potentially explaining gaps between some CI users and the best-case models.

Conclusions: Our results clarify the factors limiting prosthetically mediated hearing and illustrate a framework for predicting real-world behavioral outcomes from candidate prostheses, opening the door to large-scale screening of new device strategies.

SA54. High-Resolution Computer Models Show Neural Degeneration is the Primary Obstacle for Multipolar Cochlear Implant Stimulation

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Background: Cochlear implants electrically stimulate the auditory nerve and restore hearing to otherwise deaf individuals. Electrical stimulation, however, is broad, resulting in a small number of available independent channels. An attempt to deliver a more "focused" stimulation was the introduction of multipolar stimulation, e.g., bipolar and tripolar stimulation. In multipolar stimulation, all active and return electrodes are intracochlear, theoretically resulting in narrower, more focused electric fields. Nevertheless, multipolar stimulation has not led to advantages in speech understanding compared to the classic monopolar stimulation.

In this study, we used computer models of the human cochlea and auditory nerve to investigate why multipolar stimulation did not outperform monopolar stimulation, clarifying key constraints for future stimulation strategies.

Methods: We simulated a volume conduction model of the cochlea using the finite element analysis to obtain current spread through the inner ear, for monopolar (MP), bipolar (BP), and tripolar (TP) stimulation. The volume model was based on a high-resolution μ CT scan of a human cochlea (6 μ m voxel size). Based on the calculated current spread, we simulated the excitation of 400 spiral ganglion neuron fibers distributed along the tonotopic axis of the cochlea. Neuron models were biophysical multi-compartment models, simulated in an intact and a degenerated state. We obtained excitation patterns of the auditory nerve, and analyzed their focality and excited regions.

Results: Multipolar stimulation of intact neurons exhibited excitation not only close to the active, but also close to the return electrodes. Excitation close to return electrodes is commonly described as "side lobes". Notably, pronounced excitation gaps were found between active and return electrodes, i.e., multipolar excitation happens primarily at the electrode locations, not in between.

Regarding focality, with intact neurons, TP was generally more focal than BP and MP, despite the observed side lobes. With degenerated neurons, MP, BP, and TP were similarly focal, except that MP was comparatively more focal at high loudness levels.

Excited regions were interpreted as "place pitch". Pitch analysis was focused on cases of pitch confusion and pitch reversals. With intact neurons, pitch confusions were observed in MP and BP, while TP pitch progression was almost perfect. With degenerated neurons, MP displayed increased pitch confusions, while with BP and TP multiple pitch reversals were observed.

Conclusions: Spiral ganglion neuron degeneration is likely the main reason multipolar stimulation does not perform better than monopolar stimulation. In a perfectly intact auditory

nerve, we observed advantages of TP stimulation, which were unfortunately lost with degenerated neurons.

SA55. Assessing Spatial Hearing in Pediatric Unilateral Conductive Hearing Loss With Head-Tracking and Bone Conduction Devices

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Background: Unilateral conductive hearing loss (UHL) disrupts integration of binaural cues that underlie spatial hearing, leading to deficits in sound localization and spatial release from masking (SRM). Early auditory deprivation may result in persistent impairments in spatial processing, limiting the extent to which later amplification restores accurate binaural cue use. While implanted osseointegrated bone-conduction devices (BCDs) provide direct-drive stimulation with stable high-frequency transmission, transcutaneous non-surgical BCDs are often used in early childhood but deliver intermittent, attenuated stimulation. The impact of these differences on spatial hearing outcomes in children with UHL, remains unclear

Methods: We studied 8 children with implanted BCDs, 8 with transcutaneous non-surgical BCDs, and 8 age-matched normal-hearing (NH) peers. Participants completed a spatial speech perception task in four-talker babble with target sentences from 0° and ±60° azimuth across progressively poorer signal-to-noise ratios (SNRs). Continuous head-orienting behavior was recorded using a custom tracker. Primary outcomes included speech reception thresholds (SRTs) and reaction time to initiate head orientation toward targets. Secondary measures captured orienting dynamics, including number of head turns, corrective responses, cumulative orientation, and trial-to-trial variability.

Results: All participants showed performance decrements when signals were delivered to the impaired ear compared to the open ear or frontal targets. Implanted BCD users generally achieved better SRTs than transcutaneous users for targets on the impaired side ($p < 0.05$), though performance remained poorer than NH controls at low SNRs. Head-tracking analyses indicated NH children executed single, efficient turns with short latencies and low variability. BCD users, regardless of device type, exhibited longer reaction times, more corrective turns ($p < 0.05$), and larger cumulative head movements ($p < 0.05$), compared to NH controls. Transcutaneous users tended toward delayed initiation, increased orienting attempts, and greater overall excursion, particularly at poorer SNRs; however, the range of orienting behaviors was variable and overlapped with implanted BCD users.

Conclusions: Implanted BCDs provide more consistent stimulation and support measurable improvements in speech perception for targets on the impaired side compared with transcutaneous systems. Results also suggest that implantable BCDs support more efficient orienting behaviors compared to transcutaneous. Continuous head-tracking metrics offer ecologically valid, objective measures of spatial hearing that complement traditional audiologic outcomes. These findings highlight the role of device transmission characteristics in shaping access to binaural cues and provide insight into how different BCD approaches support spatial hearing development.

SA56. Frequency-Space Relationships in Barn Owl Auditory Pathways: Comparing Identity and Spatial Processing Streams

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Background: Previous work in barn owl midbrain has revealed a systematic relationship between frequency tuning and spatial representation in the space map: neurons representing frontal space preferentially respond to higher frequencies than neurons representing peripheral space (Shadron and Peña, 2023; Cazettes et al., 2014). This frequency-space correlation emerges within the topographic space map of ICx, where precise spatial representations are first established. However, the barn owl auditory system employs two parallel forebrain pathways: an identity pathway (ICCore, Ov, Field L) that bypasses spatial maps entirely, and a precise localization pathway (ICCore, ICCls, ICx, OT, nRt, Entopallium). Understanding whether frequency-space relationships are pathway-specific has direct implications for comprehending the functional organization of auditory processing.

Methods: We record from Field L in the identity pathway of anesthetized barn owls using multi-electrode arrays while presenting broadband and narrowband dichotic stimuli corresponding to multiple azimuthal locations by varying the interaural time difference (ITD). For each recorded unit, we quantify frequency tuning (0.5-12 kHz) and spatial receptive fields, then analyze correlations between best frequency and preferred azimuth. Population-level analyses will examine whether alternative organizational principles emerge in this identity-processing stream. Future experiments may extend recordings to the spatial processing pathway (nRt/Entopallium) to directly compare how frequency-space relationships manifest across parallel auditory streams.

Results: Since Field L receives auditory information that branches off before spatial map formation in ICx, we predict it will exhibit robust frequency tuning but lack the systematic frequency-space correlations observed in midbrain space maps. This absence would support the hypothesis that frequency-space relationships emerge specifically within topographic spatial representations rather than being a fundamental property of all auditory processing. The identity pathway's organization may reveal alternative principles for integrating frequency and spatial information that serve stimulus recognition rather than precise localization. These findings will provide insights into how different auditory pathways solve distinct computational problems and inform our understanding of how frequency-specific spatial processing deficits might arise from pathway-specific damage.

Conclusions: This research addresses a fundamental question about the functional organization of parallel auditory processing streams. By testing whether frequency-space relationships are specific to spatial map pathways or represent a general organizational principle, we will reveal how the auditory system partitions computational resources between identity and spatial processing. The predicted absence of frequency-space correlations in Field L would demonstrate that this organizational principle emerges specifically within spatial maps and serves precise localization rather than general auditory processing.

SA57. Cue Weighting in Auditory Source-Width Judgments: Implications for Binaural Cue Extraction

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Background: The spatial extent of a perceived sound, i.e., its apparent auditory source width (ASW) is known to vary with interaural coherence (IAC), or the similarity of waveforms arriving at each ear. Generally, greater IAC is associated with compact images whereas low IAC results in greater ASW, consistent with impacts of diffuse or reverberant sound. Lower IAC is also associated with greater (and more rapid) fluctuations in interaural differences of time (ITD) and level (ILD). It is not known whether the impacts of IAC on ASW relate to these fluctuations (and, if so, which ones) or to some other aspects of IAC per se. Past research suggests the importance of low-frequency IAC cues, implicating fluctuations of interaural phase difference. Here, we consider the relative weighting of ASW cues across the frequency spectrum and temporal extent of brief partially correlated noises.

Methods: Pairs of reproducible noise bursts of 100-ms duration and 0.3 octave bandwidth were presented stereophonically in the free field while manipulating the coherence of left and right audio channels. Noise bands were centered on 500, 1000, 2000, or 4000 Hz presented singly or in combination. Listeners used a graphical interface to report ASW and position judgments for each presentation. Spectral, temporal, and cross-cue weighting functions were estimated using multiple regression of judgments onto trial-by-trial variation in binaural features of each noise token extracted using simple peripheral models. Targeted features included envelope and fine-structure IAC, and dynamic ITD and ILD computed at multiple time scales.

Results: Significant variation in ASW was noted across tokens despite close matching of IAC. Token-level analyses suggest that ASW judgments reflect specific spectrotemporal dynamics of binaural cues, rather than the long-term or nominal IAC. Spectral weighting functions generally favored 500 and 1000 Hz components, consistent with a dominant role for low-frequency fine-structure cues.

Conclusions: Sensitivity of ASW judgments to temporal fluctuations of ILD and ITD, rather than IAC per se, appears consistent with known mechanisms of binaural-cue extraction supporting localization and binaural discrimination. The spectrotemporal characteristics of this process appear consistent with a dominant role of low-frequency ITD cues (as in localization), although quantitative differences in the range of frequencies and epochs involved may be noted.

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SA58. Toward the Realistic VR Sound Localization Test: From Generic Audio to Personalized Recordings

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Background: Sound localization is a core component of auditory spatial perception, relying on cues such as interaural time difference (ITD), interaural level difference (ILD), and spectral cues. However, hearing-impaired individuals such as single-sided deafness (SSD) struggle to utilize these cues effectively, leading to impaired directional hearing and challenges in daily life. Nevertheless, clinical evaluation of sound localization is rare, as it requires specialized facilities and costly equipment. This study aims to develop an optimal auditory stimulus to establish a sound localization test system based on virtual reality (VR) technology, which not only overcomes the cost and spatial limitations of traditional sound

localization evaluations but also ensures a precise assessment of hearing-impaired individuals.

Methods: 20 participants with normal hearing (NH) and 14 with SSD (7 right, 7 left) underwent sound localization tests in sound field (SF) and a VR environment. All sound localization tests used seven speakers at frontal hemifield with 30-degree intervals. Three types of noise stimuli were used: broadband (BB), low-pass with a 1,500-Hz cutoff (LP), and high-pass with a 3,000-Hz cutoff (HP). Each 500 ms stimulus (5-ms rise/fall) was presented in two spectral profiles at 55-, 60-, and 65-dB HL. In total, 126 randomized trials (18 stimuli, 7 speakers) were delivered per condition. In VR, three types of stimuli were compared: (1) Unity-based directional audio (VR audio, VRA), (2) KEMAR-recorded audio (KR), and (3) personal recorded audio (PR). Root mean squared error were compared across conditions, and the level of deficit was categorized following the ICF framework.

Results: When compared the contained spatial cues across the VR audio stimuli, PR/KR offered similar spatial cues, while VRA oversimplified and exaggerated them. In the NH group, localization error in the SF was significantly smaller than that in all three virtual conditions. Among the three virtual conditions, errors were smallest in the PR and largest in the VRA, while the KR did not differ significantly from the PR condition. SSD participants showed no significant difference between SF and VRs, with wide interindividual variability. When applying the ICF framework across 3 VR conditions, PR condition yield the most consistent result (70.58%) with the lowest error (SD: ± 0.55) comparing to the SF, demonstrating its superior reliability as a sound localization test.

Conclusions: The result of the study underscores the potential of VR tests to enhance reliability and utility in clinical and research contexts. In particular, the PR condition yielded results most comparable to those obtained from traditional sound-field localization tests, highlighting its clinical relevance and the importance of individualized auditory spatial stimuli in virtual environment.

SA59. From Innate Bias to Learned Cues: The Role of Spatial Localizability and Perceptual Learning in the Auditory Looming Bias

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Background: The auditory looming bias (ALB) describes the prioritized processing of approaching over receding sounds, supporting automatic hazard detection in dynamic environments. We previously showed that ALB is present from birth in Heschl's gyrus and persists even during inattentive listening. These findings suggest both innate and attention-independent components while leaving room for cue-specific flexibility. To probe this flexibility, we examined whether ALB depends on sound localizability and whether it can incorporate attentively learned spatial information.

Methods: In a completed study, we disrupted spectrospatial associations on the perceptual level while preserving acoustic complexity, and assessed behavioral discrimination, distance ratings, and EEG responses in comparison between native and those novel spectral cues. In an ongoing study, listeners were trained to consciously localize sounds using the novel

spectral cues, and ALB-related neural markers were collected during passive listening before and after training.

Results: Disrupting spectrospatial associations impaired localization in direction and distance and significantly altered ALB manifestation. N1 latency closely resembled distance ratings, and integrating this neural marker into a hierarchical perceptual decision-making model improved explanatory power, demonstrating a strong link between spatial localizability and ALB-related processing. For the ongoing study, we hypothesize that training will enhance ALB for novel spectral cues, such that attentively acquired localization knowledge will generalize to the sub-conscious hazard detection mechanism.

Conclusions: ALB comprises innate and attention-independent components but also relies on spatial localizability. Current work will test whether attentively learned localization cues can be integrated into this early hazard detection mechanism. Together, these studies shed light on the adaptive interplay between inborn mechanisms, automatic processing, and the flexible incorporation of learned spatial information in looming perception.

SA60. Long-Term Outcomes and Usage Patterns in Pediatric Sequential Bilateral Cochlear Implantation

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Background: Bilateral cochlear implantation (CI) has emerged as a standard approach for auditory rehabilitation in children with severe-to-profound hearing loss. However, disparities in the timing of second-side implantation (CI2) and its long-term usage patterns remain key determinants of auditory outcomes.

Methods: This retrospective cohort study analyzed 14 pediatric cases (8 female, 6 male) who underwent sequential bilateral cochlear implantation. Audiological assessments included Categories of Auditory Performance (CAP), Pure Tone Audiometry (PTA), Lexical Neighborhood Test (LNT), and Word Discrimination Scores (WDS) at 3, 6, 12, 24, 36, 48, and 60 months post-CI2. Usage duration of CI2 was categorized as: stable use (≥ 8 hrs/day), moderate use (2–8 hrs/day), or minimal use (< 2 hrs/day).

Results: The mean age at CI1 and CI2 activation was 6.0 and 13.1 years, respectively, with an average inter-implant interval of 7.1 years. Nine patients maintained stable CI2 use, while three had minimal or no usage. Patients with consistent CI2 usage demonstrated significantly higher bilateral LNT scores at 12 months (mean: 82) compared to non-users (mean: 52). Bilateral WDS at 24 and 60 months remained above 80% in stable users but declined markedly in minimal users. Longitudinal data showed improved bilateral performance over time, though CI1 consistently outperformed CI2, particularly in early months post-activation. Refusal to use CI2 was often attributed to poor subjective auditory experience or psychological resistance.

Conclusions: In conclusion, long-term bilateral cochlear implant benefit is closely associated with consistent CI2 usage. Delayed implantation and poor adherence substantially impair CI2 performance and reduce overall binaural gain. Early intervention and post-implantation counseling are crucial to maximize CI2 utility and long-term auditory outcomes in sequential bilateral CI users.

SA61. Circulating Cell-Free Mitochondrial DNA as a Predictive Biomarker for Treatment Outcomes in Sudden Sensorineural Hearing Loss

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Background: Sudden sensorineural hearing loss (SSNHL) is a common otologic emergency with variable outcomes following steroid therapy. Identification of biomarkers that can predict prognosis remains a critical unmet need. Circulating cell-free mitochondrial DNA (ccf-mtDNA) has been implicated in inflammation and tissue injury, but its role in SSNHL has not been well established. This study aimed to evaluate the association between plasma ccf-mtDNA levels, disease severity, and treatment outcomes in patients with SSNHL.

Methods: We enrolled 50 patients diagnosed with SSNHL prior to initiation of steroid therapy, along with 30 age- and sex-matched healthy controls. Plasma samples were collected on the morning before treatment. Quantitative polymerase chain reaction was used to measure ccf-mtDNA levels, expressed as log copy numbers per milliliter. Audiometric outcomes were assessed by hearing gain, percentage of recovery, and Siegel's criteria at follow-up. Multivariate logistic regression was applied to identify independent predictors of recovery.

Results: Baseline plasma ccf-mtDNA levels were significantly elevated in SSNHL patients compared to controls (mean $\pm$ SD: 8.10 ± 0.35 vs. 7.78 ± 0.65 log copies/mL; $p < 0.01$). Among patients, those with good recovery demonstrated higher pretreatment ccf-mtDNA levels than those with poor recovery (8.29 ± 0.34 vs. 8.02 ± 0.33 ; $p < 0.05$). Ccf-mtDNA levels were positively correlated with hearing gain ($r = 0.486$, 95% CI: 0.227–0.663) and percentage of recovery ($r = 0.361$, 95% CI: 0.103–0.574). Logistic regression showed that shorter duration from onset to treatment (< 7 days; OR: 7.389, 95% CI: 1.324–41.239) and higher ccf-mtDNA levels (OR: 24.634, 95% CI: 1.878–323.163) were independent predictors of favorable outcome.

Conclusions: Elevated pretreatment ccf-mtDNA levels were strongly associated with improved hearing recovery after steroid therapy in SSNHL. These findings suggest that ccf-mtDNA represents a promising prognostic biomarker that could guide clinical decision-making, allowing earlier identification of patients likely to benefit from steroid treatment. Future studies should investigate the mechanistic role of ccf-mtDNA in cochlear pathophysiology and explore its utility in tailoring individualized treatment strategies for SSNHL.

SA62. Open Board

SA63. Human Temporal Bone Histopathology in DFNB8: Findings From Two Brothers With TMPRSS3 Variants

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Background: Genetic hearing loss accounts for nearly 60% of sensorineural hearing loss cases worldwide. Mutations in the transmembrane protease serine 3 (TMPRSS3) gene cause autosomal recessive non-syndromic hearing loss, with either prelingual (DFNB10) or

postlingual (DFNB8) onset, and represent the eighth most common cause of genetic hearing loss. To date, no human histopathological data have been reported for TMPRSS3-related hearing loss. This study presents the temporal bone histopathological findings from two brothers with DFNB8.

Methods: Sixty-nine temporal bone donors with suspected genetic hearing loss, based on clinical history and with available frozen muscle (n = 40) or buccal swabs (n = 29), underwent whole-exome sequencing. Temporal bones from identified candidates were processed using standard histological techniques, followed by comprehensive histopathological analysis. Fractional hair cell survival was quantified using differential interference contrast (DIC) microscopy at 100x magnification. Supporting cell survival was evaluated using a semi-quantitative scale, and spiral ganglion neuron (SGN) counts, and three-dimensional distribution were obtained using a customized MATLAB analysis pipeline. Immunohistochemistry for TMPRSS3 protein was performed in normal control specimens to characterize its expression pattern in the human temporal bone.

Results: Two brothers were identified as compound heterozygotes for TMPRSS3 mutations, carrying the missense variants c.1276G > A (p.Ala426Thr) and c.413C > A (p.Ala138Glu). Clinically, both developed progressive hearing loss in their late twenties, which progressed to profound loss and led to unilateral cochlear implantation.

Histopathological analysis in the three available ears demonstrated poor survival of both inner and outer hair cells throughout the cochlear duct. Supporting cell survival was greater toward the apical turns but transitioned to a completely flat epithelium in the basal region above approximately 2 kHz. SGN survival differed markedly between the brothers: one exhibited reductions of 50.3% (right ear) and 56.5% (left ear) compared with age-matched controls, whereas the other demonstrated SGN counts exceeding 100% of age-matched controls.

Immunohistochemistry for TMPRSS3 performed in normal controls (n=11) showed specific labeling of Deiters' and Hensen's cells, as well as inner and outer hair cells, with particularly strong signal in the stereocilia. The outer and inner sulcus as well as the stria vascularis also exhibited specific staining. SGNs demonstrated a differential staining pattern suggesting distinct SGN populations.

Conclusions: This study provides the first human temporal bone histopathological description of TMPRSS3-related hearing loss. The findings reveal widespread hair cell loss, variable SGN survival between individuals with the same genotype, and distinct TMPRSS3 protein localization within cochlear supporting cells, hair cells, and SGNs. These results expand the understanding of the cellular pathology associated with DFNB8 and may inform future therapeutic strategies.

SA64. Automatic Scoring of Speech in Noise Tests

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Background: Measurement of speech recognition abilities is crucial given that the top complaint of individuals with hearing difficulties is understanding speech in noise. However, there are several limitations to the standard approach used in clinical and research settings. First, testing patients in a non-native language is problematic. Second, human scorers may be biased towards the expected words. Finally, audiologist-driven scoring limits the number of

individuals we can test. These issues can be addressed using automatic speech recognition (ASR); and are now feasible given advances in AI and speech-recognition systems.

Methods: We implemented 6 tests of speech recognition (CNC, QuickSIN, WIN, and AzBio in varying signal-to-noise ratios (SNR)). A tester played the sounds and scored the tests in real time. Spoken utterances were stored for later analysis. We tested 28 native English subjects, half of whom had normal hearing thresholds. The remaining individuals all had bilateral sensorineural hearing loss. We scored 4310 participant responses. Each response was converted to text using the Whisper AI Large Recognizer (v20250625). We scored each answer by looking for exact textual matches, regardless of their position or grammatical issues.

Results: We found that human and machine recognition agreed ~93% of the time for the multi-word tests (QuickSIN, AzBio) and ~81% of the time for the single word tests (CNC, WIN). This correspondence was similar across varying degrees of hearing loss and SNR values. In a random sample of 100 discrepancies, we found that 9 times the tester was incorrect, and 37 times the ASR system transcribed the answer incorrectly. There were three other types of errors, which we now realize were due to our experimental setup and thus correctable. These include 10 times when the ASR engine switched languages, 32 times when the recognizer produced a homonym or a variant of the word we were not expecting (5 vs. five). And 12 times when there was a recording issue and we should have asked the subject to respond again.

Conclusions: These results demonstrate that Automatic Speech Recognition is a feasible approach for scoring audiologic tests of speech recognition in quiet and noise in patients with normal hearing or hearing loss. This approach can enable open-set speech recognition tests measured online or remotely, and may therefore have many clinical or research applications. Going forward we want to 1) better characterize the discrepancies between machine and human scoring, 2) investigate the recognizer's biases, and 3) develop open-source testing material across a range of languages that we see in our clinic. In this way we can ensure that online testing of open-set speech materials is both robust and repeatable.

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SA65. Specialty-Specific Symptom Profiles for Vestibular Schwannomas: Insights From NLP Analysis of Otolaryngology and Neurosurgery Clinical Notes

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Background: Vestibular schwannomas (VS) patients commonly present with hearing loss, tinnitus, dizziness, and imbalance. Both ENT and neurosurgery (NSG) evaluate VS, and documentation emphasis may differ in ways that reflect specialty-specific evaluation and management. This study seeks to compare specialty-specific documentation patterns using Natural Language Processing (NLP).

Methods: We compiled 982 de-identified clinical notes for 739 patients with first-time clinic visits to ENT (n=412) or NSG (n=570) at a tertiary academic medical center between 2014-2024. Notes were cleaned and pre-processed using stop words and Regex rules to filter out non-first-time clinic visits and templated history and physical exam findings. Note text was embedded with a Clinical BERT transformer model to obtain fixed-length semantic vectors, and a low-dimensional structure was visualized with t-Distributed Stochastic Neighbor

Embedding (t-SNE). Discriminability of specialties in the original embedding space was evaluated with logistic regression (AUC). Vocabulary divergence was quantified with bootstrap Jensen-Shannon distance (JSD). Clinically relevant bi-grams and tri-grams were extracted, and specialty frequency differences were tested with a two-proportion Z-test.

Results: Embedding revealed strong specialty-specific documentation patterns. In 2-D t-SNE, notes exhibited partial provider clustering with overlap; global separation was modest (silhouette score 0.05, centroid Euclidean distance 1.10; $p < 0.001$). In contrast, a logistic regression on all embeddings distinguished ENT vs NSG with near-perfect discriminability (AUC=0.998). Vocabulary distributions differed substantially (bootstrap JSD 0.62, 95% CI 0.59-0.65, $p < 0.001$). Shared high-frequency symptoms included hearing loss (ENT:2.88 mentions/note, NSG:1.12), sensorineural hearing loss (ENT:0.16, NSG:0.13), vertigo/dizziness (ENT:0.20, NSG:0.12), facial nerve weakness (ENT:0.14, NSG:0.17), facial numbness (ENT:0.35, NSG:0.26), tumor growth (ENT:0.44, NSG:0.05), and family history (ENT:0.38, NSG:0.18). After FDR correction, mentions of tumor growth, family history, likely/symptomatic hearing loss, vertigo/dizziness, and facial numbness differed significantly between specialties ($p < 0.005$). Additional phrases more unique in ENT notes include history of ear infections (0.29); hearing aid (0.28); diplopia (0.13). Phrases more frequently documented in NSG notes included tinnitus (0.32), ability/inability to use phone (0.29), balance issues (0.23), and facial pain (0.11).

Conclusions: Despite modest low-dimensional clustering, high-dimensional NLP features and phrase distributions reveal reproducible specialty-specific documentation differences between ENT and NSG VS notes. ENT documentation more often emphasizes hearing-related concerns, multi-systemic symptoms, and tumor growth, whereas NSG notes more frequently refer to facial nerve-related symptoms, tinnitus, and functional/quality of life issues. These results characterize documentation patterns and may reflect referral pathways, patient presentation, and specialty emphasis. Future studies should evaluate NLP-based tools to support referral routing and terminology that may better facilitate multidisciplinary care coordination for VS patients.

SA66. Chronic Rhinosinusitis in Non-Solid Transplant Recipients

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Background: Non-solid transplants, which includes bone marrow transplants (BMT) and hematopoietic stem cell transplants (HSCT), are becoming increasingly used to treat hematological and autoimmune conditions. Several characteristics of this population make them more susceptible to developing chronic rhinosinusitis (CRS) after transplantation, most notably the use of immunosuppressive medications. However, there is a lack of robust studies evaluating the incidence of CRS in non-solid transplant populations and other related characteristics. Therefore, our aim is to understand the extent and type of evidence available in relation to the incidence, clinical characteristics, and management of CRS in non-solid transplant recipients.

Methods: A systematic review was conducted to identify studies describing CRS in non-solid organ transplant recipients. Databases searched included Ovid Medline, Ovid Embase, Pubmed, Scopus, Web of Science, Cochrane, and Google Scholar. Keywords and medical subject headings that were searched included chronic rhinosinusitis, rhinosinusitis, sinusitis, bone marrow transplant, and stem cell transplantation. The primary objective was to analyze

the current literature regarding the prevalence of CRS in this population. The secondary objective was to characterize the clinical presentation of CRS among these patients.

Results: A total of 2735 studies were identified and 1970 studies were screened. From these, 44 studies full-text studies were assessed for eligibility. From these, 14 studies met the inclusion criteria. Of the 14 manuscripts, there was 1 case-control and 13 cohort studies. A total of 1356 transplant recipients were identified, with 312 of the recipients developing CRS, resulting in a pooled prevalence of 23.0%. The most common underlying hematological malignancies included acute myelogenous leukemia (AML, 19.02%), acute lymphocytic leukemia (ALL, 9.66%), and chronic myelogenous leukemia (CML, 7.08%).

Conclusions: Individuals receiving non-solid transplants are at a significantly greater risk of developing CRS than the general population, with an incidence of 23.0%. Individuals with underlying AML seem to represent a particularly susceptible subtype of hematological malignancy. Further investigation is warranted to identify predictive factors and high risk subgroups related to CRS development in this population.

SA67. A Study on Food Intake Level Scale and Rehabilitation Interventions Following Aspiration Prevention Surgery at Our Hospital Evaluation of the Food Intake Level Scale and Rehabilitation Interventions Following Aspiration Prevention Surgery at Our Institution

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Background: Surgery for preventing aspiration is a reliable method for preventing aspiration in patients with severe dysphagia. It can improve patient quality of life (QOL) by offering the possibility of resuming oral intake postoperatively. At our institution, we actively implement swallowing rehabilitation by speech-language pathologists (SLPs), nutritional support by registered dietitians, and multidisciplinary interventions by the swallowing team to promote postoperative swallowing function recovery. However, there are few reports on the relationship between specific intervention methods and improved feeding status following anti-aspiration surgery.

Methods: This study included 44 patients aged 18 years or older who underwent aspiration prevention surgery in our department between January 2014 and December 2023. Pediatric cases were excluded due to underlying conditions and difficulties in intervention. Eating status was assessed using the FILS (Food Intake Level Scale) at admission for preoperative status and at discharge for postoperative status. Factors associated with FILS improvement pre- and postoperatively were analyzed univariately. For factors with $p < 0.01$, multivariate logistic regression analysis was performed adjusting for age and gender. Additionally, ROC curve analysis was performed for the number of ST interventions to calculate a cut-off value.

Results: FILS improvement was observed in 29 cases (65.9%) between pre- and postoperative periods. Additionally, 29 cases (65.9%) achieved a postoperative FILS of 4 or higher, enabling oral intake. Cases with lower preoperative FILS showed significantly greater improvement ($p < 0.05$). The group receiving ST intervention showed significantly improved postoperative FILS, with the improvement rate increasing with the number of interventions. ROC analysis indicated a cut-off value of 0.5 interventions (≥ 1 intervention) for the number of ST interventions. OT intervention and the number of postoperative rehabilitation sessions were also suggested to be associated with improvement.

Conclusions: The presence and number of ST interventions were the most important factors for improving feeding status after dysphagia prevention surgery. Improvement is possible even in cases with low preoperative FILS scores, making proactive multidisciplinary intervention desirable. Further investigation is needed on the effectiveness of comprehensive team interventions, including OT intervention.

SA68. Establishing an in Vivo Model for Stem Cell Based Treatment of Laryngotracheal Stenosis

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Background: Laryngotracheal stenosis is a common complication of prolonged mechanical ventilation with devastating consequences on a patient's quality of life. Despite this, there remain no effective treatments to prevent or reverse it. The advent of stem cell-based therapeutics presents a promising treatment option for airway injury. We have developed a novel strategy to produce lung epithelium basal cells in vitro (iv-BCs) from human pluripotent stem cell derived lung organoids (hPSC-LPs). Furthermore, we have demonstrated in previous experiments the survival, proliferation and differentiation of hPSC-LPs into various airway epithelial cell types when seeded onto de-epithelialized mouse and human tracheas cultured ex vivo. We believe that stenosis forms when the injured airway epithelium fails to repair in time, allowing underlying fibroblasts to react to the injury and secrete excess extracellular matrix, ultimately leading to scarring and narrowing of the airway. We hypothesize that if iv-BC or hPSC-LPs are applied to the site of injury, they will function like a "biologic band-aid," promoting rapid epithelial repair and thereby preventing or reducing the degree of stenosis. As a critical next step towards establishing translational clinical trials, we aim to demonstrate the same successful engraftment, proliferation and differentiation of hPSC-LPs in vivo using a medium-sized animal model, a proof of concept study.

Methods: We obtained 10 male New Zealand white rabbits to undergo surgery to induce short segment laryngotracheal stenosis. The surgery consists of a 3 cm longitudinal incision at the neck and blunt dissection through the neck fascia to access the trachea, followed by an incision cutting approximately two-thirds of the tracheal circumference in order to access the lumen. A 5.5 mm steel brush is then inserted into the tracheal lumen and brushed back and forth five times across the luminal mucosa to damage the epithelial layer and induce stenosis. The trachea is closed, the rabbits allowed to recover until 21 days post-operation. The rabbits are then sacrificed and their tracheas are dissected. The trachea is paraffin embedded and later stained via hematoxylin and eosin (H and E) to determine the extent of stenosis and whether this is standardized across rabbits.

Results: We observed successful laryngeal stenosis in all of our rabbit models. This is denoted by an enlarged lamina to cartilage ratio measured in all of our surgically injured rabbit tracheas as compared to our control, uninjured tracheas.

Conclusions: The success of our medium animal stenosis model is an essential first step in achieving our ultimate goal of demonstrating the successful engraftment and proliferation of hPSC-LPs in vivo in rabbit models. Future directions are aimed at producing the same injury in immunodeficient rabbits with the added engraftment of hPSC-LPs at time of injury. This will be followed by similar histological analysis.

SA69. Potassium Channels Underlying Serotonergic Excitation of Medial Olivocochlear Efferent Neurons

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Background: Activation of auditory efferent neurons directly modulates cochlear sensitivity to sound. Axons of cholinergic medial olivocochlear neurons (MOCs) located in the ventral brainstem directly target outer hair cells (OHC), hyperpolarizing them to reduce the effect of cochlear gain. We have previously shown that exogenously applied serotonin (5-hydroxytryptamine or 5-HT) affects intrinsic response properties of MOC neurons, increasing their excitability, consistent with the idea that serotonin may be implicated in protection from acoustic trauma. Here, we explore the role of different potassium channels expressed in MOC neurons mined from previously published nucSeq data (Frank et al., 2023) to investigate the contribution of potassium currents to this serotonin mediated excitation of MOC neurons.

Methods: In vitro patch clamp recordings were made from cholinergic MOC neurons from ventral brainstem slices of ChAT-IRES-Cre;Ai14 mice (n = 56) in current clamp configuration. Pharmacological experiments were designed to block specific potassium channels before and during bath application of 100uM serotonin. Specific channel targets were: apamin to block small conductance calcium-activated potassium (SK) channels (100nM), iberiotoxin to block big conductance calcium-activated potassium (BK) channels, XE-991 to block Kv7 channels (1μM), guangxitoxin-1E to block Kv2 channels (100nM), phrixotoxin-2 to block Kv4 channels (200nM), and tertiapin-Q to block g-protein coupled inwardly rectifying (GIRK) channels (100nM).

Results: Pharmacology experiments confirmed the presence of multiple functional potassium channels in MOC neurons. The excitatory effects of serotonin on MOC neurons—measured using firing rate in response to 100pA of injected current—persisted in the presence of all potassium channel blockers tested, apart from apamin (SK channels) and XE-991 (Kv7 channels). The serotonin mediated increase in MOC neuron input resistance was occluded in the presence of apamin, iberiotoxin and phrixotoxin-2, implicating both calcium-activated (SK and BK), and Kv4 channels. Together, these results suggest that the mechanism underlying serotonergic excitation of MOC neurons may be tied to calcium ions: SK and BK channels are calcium gated, and the Kv4.2 subunits and Kv7.1 subunits (both enriched in MOCs), are reported to interact with multiple calcium channels and calcium-binding proteins, respectively.

Conclusions: In conclusion, we found that potassium conductance through SK, BK, Kv4 and Kv7 channels contribute to serotonin mediated increases in MOC neuron excitation in vitro. Because increasing MOC activation attenuates cochlear responses to loud sounds, it is tempting to speculate that serotonin mediated excitation could be a mechanism of providing context dependent efferent control of the cochlea. Further in vivo experiments are necessary to understand the functional significance and circumstances under which this neuromodulation of MOC neurons contributes to auditory processing.

SA70. A Population of Monaural Brainstem Neurons Projecting Into the Lateral Lemniscus Shows Enhanced Temporal Synchronization to Stimulus Fine-Structure

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Background: Enhanced synchronization to the temporal components of sound, relative to the auditory nerve, is well documented for several cell types in the cochlear nucleus. Prominent examples are bushy cells (for encoding the temporal fine-structure of low-frequency sounds) and octopus cells (for encoding onsets and envelopes). Similar but less well documented behaviors are found in the subcollicular targets of these neurons.

Methods: We used a new dorsal surgical approach close to the DNLL to record from axons in the lateral lemniscus (LL). Binaural and monaural stimuli were presented through speakers directly coupled to the bulla. Each axon was studied with stimuli traditionally used to classify monaural and binaural responses. Axonal penetration and injection of biocytin was then attempted to morphologically identify single neurons that generated responses of interest.

Results: Although the cell types projecting into the LL are reasonably well documented, there was a clear recording bias towards certain response types likely reflecting axonal bundling and axon size. Unexpectedly, we frequently encountered monaural neurons with “hi-sync” responses to contralateral low-frequency tones, whose degree of temporal enhancement surpassed that of bushy cells recorded in the same species. Neurons showed “entraining” (i.e. phase-locked entrainment) for tones up to ~ 600 Hz. Responses to broadband noise were enhanced as well, as assessed with autocorrelograms. In only one case was an axon successfully injected: while the parent cell body could not be found, the labeled axon had a large diameter.

Conclusions: Because bushy cells do not project as far dorsally in the LL as our recording site, we surmise that there is a source of monaural neurons somewhere in the superior olivary complex generating highly synchronized spiking which is relayed to rostral and dorsal levels in the midbrain. Cells or their axons showing such behaviors have been poorly sampled in previous (extracellular) studies of the superior olivary complex, but are important to consider and further study to help clarify the mechanisms underlying binaural temporal sensitivity and pitch.

SA71. Imaging Unipolar Brush Cells Using Fluorescently Tagged Fabs and Nanobody Probes

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Background: Unipolar brush cells (UBCs) of the vestibular cerebellum and dorsal cochlear nucleus are excitatory interneurons that transform synaptic signals from mossy fiber terminals into a complex pattern of spikes and convey these signals to postsynaptic granule cells and to other UBCs. The contact between mossy fibers and the UBC brush dendrite is a complex structure that appears to retain glutamate for relatively long periods compared to conventional synapses, thus prolonging synaptic signals. These signals are mediated by ionotropic (AMPA) and metabotropic (mGluR) glutamate receptors. We sought to examine these contacts using confocal microscopy.

Methods: We used GRP-cre x Ai9 mice in which tdTomato was expressed in the ON subtype of UBC. Postsynaptic receptor clusters were labeled using a GFP-tagged antibody fragment (Fab) directed against an extracellular portion of the GluA2 subunit of the AMPA

receptor, the dominant subunit identified with RNAseq of UBCs. Efficient labeling required incubation of unpermeabilized, unfixed, living brain slices in the Fab. Following 30-min incubation, slices were rinsed, fixed, permeabilized and incubated overnight with an Atto643-tagged nanobody directed against Vglut1 to label presynaptic terminals. Slices were then rinsed, mounted in Fluoromount-G and imaged with a Zeiss 980 using Airyscan deconvolution.

Results: This approach revealed receptor clusters and presynaptic terminals with low background fluorescence. The absence of secondary antibodies enabled faster processing than with conventional immunohistochemistry and likely contributed to the low background. Most clusters were clearly positioned adjacent to presynaptic terminals. Some clusters were positioned on the surface of the brush dendrite while others were not; the latter were likely receptors at mossy fiber-granule cell synapses. These presumptive granule cell synapses (not associated with the UBC) were the majority of clusters in each image and appeared as puncta of ~ 0.2 micron diameter, at the limit of the PSF. Notably, clusters on the UBC dendrite were clearly different in shape and size from the puncta, with many appearing as extended, branched fingers over 1.5 micron long, in keeping with previous EM observations of synaptic densities.

Conclusions: Two striking take-home observations were 1) although UBC postsynaptic sites were large, there were not many of them. Most of the dendrite surface was apparently not devoted to fast transmission, suggesting additional functions to the brush dendrite. 2) The brush dendrite wrapped around mossy fiber synapses and receptors that appeared to be not synaptically associated with the brush, suggesting that the brush enshrouds granule cell claw dendrites. We speculate that there may be a functional difference between mossy-fiber/granule cells contacts confined by the UBC brush and those unconfined, perhaps differing in the retention of transmitter.

SA72. State-Dependence of Cell-Type Specific Circuit Interactions in the Dorsal Cochlear Nucleus

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Background: Activity in neuromodulatory systems fluctuates continuously during wakefulness, influencing neural processing broadly across the brain. While the effects of brain state in the auditory cortex and thalamus have been extensively characterized (e.g. McGinley et al., 2015), its effects on earlier stages of auditory processing remain poorly understood. For example, the dorsal cochlear nucleus (DCN) is an early brainstem structure that integrates auditory with non-auditory inputs to flexibly localize sounds, representing a critical site for investigating how internal brain state shapes sensory encoding.

Methods: Here, we recorded single-unit activity from the DCN of awake, head-fixed mice using high-density Neuropixels probes. We simultaneously tracked pupil diameter and locomotion as proxies for neuromodulatory brain state, as well as ear kinematics due to their importance in sound localization. DCN neurons were classified into putative cell types: putative principal cells and their response subtypes, or cartwheel or vertical cell (the two main classes of DCN inhibitory interneurons), based on their responses to tones and broadband noise and with optogenetic photo-tagging.

Results: We found that spontaneous firing rates exhibited robust, cell-type specific modulation by pupil-indexed arousal level. Principal neuron subtypes that were excited at their BF (type III) or exhibited complex tuning (type IV) showed monotonically increasing firing rates with pupil size, with much stronger modulation than the subtype inhibited at BF (type IIIi). Cartwheel cells also displayed robust state dependence, whereas vertical cells, due to their minimal spontaneous activity, showed no state dependent modulation. Moreover, neuronal synchrony in the DCN varied systematically with pupil size in a cell-pair type-specific manner: cartwheel cells exhibited a U-shaped relationship, whereas principal neuron subtypes showed a monotonic increase in synchrony with pupil size, suggesting that internal state not only modulates individual neuron excitability but also affects circuit-level interactions.

Conclusions: In contrast to downstream cortex and thalamus, the effects of pupil size on sound-evoked responses in DCN were comparatively small, suggesting fundamentally different principles govern state-dependence DCN compared to e.g. thalamo-cortical circuits. Furthermore, we find that the mice exhibit several types of ear movements, including orienting, twitch, perking, and wiggling, each with distinct relationships to pupil-indexed state. These ear movements have cell type-specific effects on DCN activity and sound location decodability. Together, these findings highlight the DCN as a critical node in auditory system state-dependent auditory processing.

SA73. Measuring Meaningful Outcome for Adult Hearing Health Interventions

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Background: The pure-tone audiogram is the clinical gold standard for adult hearing assessment. However, speech is a complex acoustic signal and the audiogram does not directly capture communication difficulties. Moreover, restoring audibility does not assure communication challenges resolve. When assessing functional benefits associated with use of a drug or device, researchers and clinicians have used a variety of different outcome measures to supplement the audiogram. However, even if outcomes beyond the audiogram are measured, the tools used to measure these outcomes vary across studies, clinicians, and patient encounters.

Methods: To work towards meaningful outcome measurements, the U.S. National Institutes of Health, Defense Health Agency, and Veteran's Administration commissioned a report from the National Academies of Science, Engineering, and Medicine (NASEM). An ad hoc committee was assembled by NASEM. The committee was tasked with identifying domains that are important across patients, settings (clinic, research), and interventions (hearing aids, pharmaceuticals, biologics, auditory training). The committee examined the state of the science in outcomes research for interventions in adult hearing healthcare (excluding surgically placed prosthetic devices), with an emphasis on measures that are meaningful to the individual and the clinician.

Results: The NASEM committee report, "Measuring Meaningful Outcomes for Adult Hearing Health Interventions," was released May 7, 2025. The report recommends a core set of outcome domains with corresponding measures. The committee came to consensus on two recommended outcome domains: understanding speech in complex listening environments and hearing-related psychosocial health. Recommended measurements include the Words-in-

Noise (WIN) test and Abbreviated Profile of Hearing Aid Benefit (APHAB) global score (speech understanding), and the Revised Hearing Handicap Inventory (RHHI) (hearing-related psychosocial health). These outcome measures are recommended across settings (clinic, research), indications (age-related hearing loss, noise-induced hearing loss, etc.), and interventions (hearing aids, pharmaceuticals, etc.). The report notes that supplemental measures may be needed; these are a minimum recommended standard data set for those measuring patient and participant outcomes.

Conclusions: The current lack of standardization limits direct comparisons across data sets and is an obstacle to the development of big data approaches within hearing health intervention research. Big data approaches require data repositories. Open data requirements increase the urgency of the need for data repositories to be both available and used. The authors invite meeting attendees to discuss the report from both the sponsor perspective and the committee perspective.

SA74. Using Click Interaural Time Difference Stimuli to Characterize Excitatory and Inhibitory Inputs to the Inferior Colliculus in Awake Rabbit

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Background: Responses to interaural time differences (ITDs) of click stimuli in the inferior colliculus (IC) of anesthetized cat were used to characterize the dynamics of excitation and inhibition from ipsilateral and contralateral sources (Carney and Yin, 1989, *J. Neurophysiol.*, 62:144-161). These results informed IC model parameters and contributed to our understanding of differences between responses to diotic and contralateral stimuli. However, IC responses differ between anesthetized and awake animals, and responses to click ITD have not been recorded in awake animals. Here we determined the timing and strength of inhibition and excitation of IC neurons by recording click-ITD responses in awake rabbit.

Methods: Single-neuron responses were recorded using tetrodes in the central nucleus of the IC in awake Dutch-belted rabbits. Click rate-level functions were created by presenting 100- μ s clicks to the ipsilateral or contralateral ear at sound levels 30-100 dB peak-equivalent sound pressure level (dB peSPL). Click ITD stimuli with 100- μ s duration were presented with interaural delays from 0 to $\pm$ 50 ms, in increments of 1 ms. Twenty repetitions of each stimulus were presented in random order. Sound levels were chosen to ensure suprathreshold responses, based on the rate-level function, and spanned 53-83 dB peSPL. Contralateral and ipsilateral contributions to the click-ITD response were determined using alpha functions to estimate post-synaptic potentials for excitation, inhibition, and facilitation.

Results: IC neurons had consistent and robust responses to clicks. Most often, the contralateral click elicited strong excitation, and the ipsilateral click elicited weak excitation followed by stronger inhibition. Facilitation, when a contralateral click increased the response of a following ipsilateral click, was also observed. However, neurons showed a wide variety of responses to stimuli depending on the timing and strength of these inputs. Most neural responses fell into four categories: 1) Reduction in rate near zero delay and increase in rate at other delays resulting from contralateral excitation and contra/ipsi inhibition, 2) reduction in rate near zero delay with an increase in rate at other delays resulting from additional facilitation components, 3) increase in rate at zero delay and suppression at other delays and 4) weak inhibition or excitation near zero delay. Additionally, the spontaneous rate of IC

neurons was often high enough to observe inhibition, which differed from the anesthetized cat study.

Conclusions: These results identified and characterized several different time courses of ipsilateral and contralateral excitatory and inhibitory inputs that influence binaural sound encoding in the awake IC. Results may improve computational IC models and explain differences in responses to complex stimuli presented diotically or contralaterally. Supported by: NIH R01-DC010813, NIH F31-DC020630

SA75. A Molecular Roadmap for Central Auditory Projections

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Background: All auditory signals from the cochlea are transmitted via the auditory nerve to the cochlear nucleus (CN), the first central hub for auditory processing. Within the CN, these signals are integrated and relayed to higher brain regions for auditory perception, sound localization, aversive responses, and integration with circuits mediating emotional salience and memory. However, the precise diversity of CN neuronal subtypes and the specificity of their long-range projections remain poorly understood.

Methods: We leveraged virus-based retrograde labeling and deep single-nucleus RNA sequencing to comprehensively map CN neuronal diversity and link transcriptomic cell types to their projection targets. In parallel, we are performing electrophysiological recordings to correlate CN molecular subtypes with their physiological properties and using rabies virus tracing from downstream brain circuits to test for selective “labeled line” pathways.

Results: Our approach uncovered substantial molecular and biological diversity among CN populations with distinct anatomical projections, even when they form a single genetic cluster. We established a unified transcriptomic and projectional taxonomy of CN neurons across functionally distinct auditory pathways, revealing organizational principles of the CN.

Conclusions: This work defines how molecularly diverse cochlear nucleus neurons contribute to distinct auditory pathways. It provides a framework to understand the organization and routing of auditory information in the brain, which will help dissecting the mechanisms of early auditory processing.

SA76. Vasopressin and Oxytocin Distribution in the Auditory Brainstem of Rodent With Diverse Social Structures

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Background: The nonapeptides vasopressin (AVP) and Oxytocin (OT) play critical roles in regulating social behavior across diverse taxa. Their distribution has been well documented in the forebrain and midbrain, revealing relatively strong evolutionary conservation. However, their distribution throughout the hindbrain remains poorly understood.

Methods: Here, we used immunohistochemical techniques to quantify AVP and OT cells' distribution in the superior olivary complex of the brainstem - specifically the lateral superior olive (LSO), the medial superior olive (MSO), and the medial nucleus of the trapezoid body (MNTB) across six wild rodent species with distinct social structures. The species examined represent solitary (*Chaetodipus hispidus* and *Dipodomys ordii*), monogamous (*Microtus*

ochrogaster and *Onychomys leucogaster*), and group-living (*Mus musculus* and *Rattus norvegicus*) social systems. We also measured overall size and structural differences in these auditory brainstem regions among species.

Results: We predicted that group-living species will exhibit higher distribution of AVP and OT cells in the LSO, MSO, and MNTB compared to monogamous or solitary species. We also predicted that group-living species will have larger LSO, MSO, and MNTB areas than other measured groups.

Conclusions: These findings will provide a comparative mapping of AVP and OT cells' distribution in the rodent brainstem and offer a foundation for understanding nonapeptide-mediated behavior in species with varying social structures.

SA77. Parallel Auditory Brainstem Response (pABR) Accurately Predicts Air- And Bone- Conduction Thresholds in Adults

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Background: Frequency-specific auditory brainstem responses (ABRs) are essential for estimating hearing thresholds when behavioral testing is not feasible. The parallel ABR (pABR) has been shown to speed up testing by presenting multiple frequencies to both ears (Polonenko and Maddox, 2019), and an in-progress study suggests it provides accurate estimates of hearing thresholds in adults with sensorineural hearing loss. Up to now, work on the pABR has focused on air-conducted stimuli. However, validating bone-conducted stimuli is important as they allow clinicians to distinguish between types of hearing loss. Therefore, in this study, we extended the pABR to include both air- and bone-conduction and evaluated its accuracy in estimating thresholds and classifying both the type and degree of hearing loss.

Methods: Two-channel ABRs were recorded from 40 adults (11 men, 29 women; aged 30–70 years) with varying audiometric configurations: 20 with sensorineural hearing loss and 20 with conductive or mixed losses. Stimuli consisted of 0.5, 1, 2, 4, and 8 kHz tonebursts presented to both ears for air-conduction testing and 0.5, 1, 2, and 4 kHz tonebursts presented monaurally for bone-conduction testing, with contralateral masking (pABR stimuli presented at a faster rate) delivered to the non-test ear. ABR wave V responses were identified online during testing and compared with behavioral thresholds obtained after the pABR testing in the same session.

Results: pABR testing produced accurate threshold estimates for both air and bone conduction. For air conduction, 85% of pABR-estimated thresholds (without correction factors applied) were within 10 dB, and 96% within 15 dB of behavioral thresholds, with a very strong correlation between predicted and behavioral thresholds ($r = 0.92$, 95% CI: 0.90–0.93, $p < 0.001$). For bone conduction, 77% of pABR-estimated thresholds were within 10 dB and 91% within 15 dB of behavioral thresholds, also with a strong correlation between thresholds ($r = 0.75$, 95% CI: 0.69–0.80, $p < 0.001$). Testing was also efficient, requiring a median of 65 minutes to collect all 18 thresholds (~3.6 minutes per threshold), regardless if the loss was purely sensorineural or had a conductive component (median times of 64 and 68 minutes, respectively). These findings suggest that the pABR can efficiently test thresholds without sacrificing accuracy.

Conclusions: The pABR provides fast, accurate, and reliable threshold estimates across air and bone conduction testing, allowing accurate classification of both the type and degree of hearing loss. These findings highlight the clinical potential of pABR to improve diagnostic

efficiency and inform treatment decisions. Future work will evaluate its performance in infants and young children, where objective, rapid threshold estimation is most critical.

SA78. From Sound to Movement: The Neural Backbone of the Acoustic Startle Reflex

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Background: The acoustic startle reflex (ASR) is a fast bodily reaction to a loud and unexpected auditory stimulus – a protective mechanism inherent to most mammalian species. On cellular level, large cochlear root neurons (CRNs), located between the auditory periphery and cochlear nucleus, have been identified as the first neural substrate of the underlying reflex circuitry. CRNs receive glutamatergic input from primary afferent spiral ganglion neuron (SGN) collaterals and were shown to be subject to various efferent modulatory pathways. Their large axons mainly target neurons of the contralateral pontine reticular nucleus (PnC) formation, which project onto cranial and spinal motor neurons and are in turn subject to extensive modulatory inputs from various brain areas. Despite their fundamental role in a survival reflex circuit, CRNs are still remarkably poorly characterized in regard to their cellular excitability and activity patterns as well as their molecular composition and morphology in situ. Despite behaviorally apparent acoustic startle reflexes in humans, the taxonomic equivalent of rodent CRNs has yet to be identified. However, ASR measurements are of major clinical relevance, as altered startle behavior is linked to neuropsychiatric or neurodevelopmental disorders – such as autism spectrum disorders.

Methods: Here, we prepared fixed vibratome-sectioned tissue slice preparations of the cochlear root nucleus as well as whole-mount preparations of the intact peripheral auditory pathway of mice (*Mus musculus*) for immunohistochemical stainings and ethyl cinnamate based tissue clearing. For fiber input tracing experiments, we utilized various genetic reporter mouse models alongside lipophilic tracer dyes. Data were acquired using confocal and lightsheet fluorescence microscopy and analyzed using Imaris software. To probe evolutionary conservation and the functional role of CRNs in various animal models with distinct hearing frequency ranges, we additionally analyzed Mongolian gerbils (*Meriones unguiculatus*), Etruscan shrews (*Suncus etruscus*) and common fruit bats (*Carollia perspicillata*).

Results: In this work, we were able to develop a novel tissue preparation technique that preserves the cochlear root nucleus within its native context in the intact peripheral auditory pathway. Lightsheet images of such chemically-cleared preparations allowed us to characterize the exact 3D localization, morphology, size and count of the CRN population. Furthermore, we analyzed the afferent and efferent synaptic connectome of CRNs to establish excitatory and inhibitory neurotransmitter identities as well as their respective innervation densities and further plan to probe their role on cellular excitability measures.

Conclusions: In this project, we aimed for a holistic in situ characterization of the CRN population by performing detailed anatomical, morphological and functional analyses regarding cellular location and neuronal connectivity. Our current findings yield strong evidence for distinct CRN subpopulations that may serve differential functions in the ASR

pathway – thereby highlighting the surprising complexity of a supposedly simple and rapid reflex circuit.

SA79. Evidence for a Vestibular Contribution to Auditory Processing via the LVN–IC Pathway

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Background: The vestibular nuclei (VN) are brainstem structures essential for balance and posture. They play a key role in natural behavior by broadcasting vestibular signals across the brain and interacting with other sensory systems. When this multisensory coordination is disrupted, neurological disorders may occur, often resulting in combined vestibular and auditory symptoms. However, the specific contribution of the VN to auditory processing remains unclear. Clarifying how the VN connects to the inferior colliculus (IC) and what functional influence this connection may provide insight into this question. Accordingly, this study aims to investigate whether the VN and IC are anatomically and functionally connected.

Methods: C57BL/6J mice (7–9 weeks old) were anesthetized and injected with CTB-Alexa568, anterograde AAV, or retrograde AAV into the IC or lateral vestibular nucleus (LVN). For electrophysiology, mice were implanted with a headpost before recording. Two small cranial windows were then made over the IC and LVN for neural recording and optogenetic stimulation. Neural signals were acquired using a 6-channel tungsten electrode array (~5 MΩ, FHC). To assess sound responses, animals were placed in a sound booth and exposed to 70 dB pure tones (8 and 32 kHz) for 2 hours following 90 minutes of silence. Brains were fixed, sectioned, and processed for c-Fos immunostaining to verify sound stimulation-evoked activity.

Results: To explore the connectivity between the LVN and IC, we used viral tracing. Retrograde virus injection into the IC resulted in labeled neurons in the LVN. Anterograde virus injection into the LVN revealed labeled fibers in the ventromedial region of the IC. Retrograde virus injection into the LVN led to labeling in the uvula and cochlear nucleus (CN). Double-labeled neurons expressing both retrograde labeling from the IC (mCherry) and anterograde labeling from the uvula (GFP) were identified in the LVN. We performed optogenetic stimulation of the LVN. Subsequent electrophysiological recordings from the IC revealed strong short-latency modulation of IC neuron firing rates. To test whether IC-projecting LVN neurons respond to auditory stimuli and vestibular input, we conducted a sound exposure experiment. After sound stimulation, c-Fos expression was detected in the LVN in response to 8 and 32 kHz sounds.

Conclusions: Our findings demonstrate both anatomical and functional connectivity between the LVN and the IC. These results suggest that the LVN, in addition to its role in balance control, also contributes to auditory processing, thus providing new evidence for how vestibular and auditory functions are integrated through the LVN–IC pathway.

SA80. Inhibition of Extracellular Vesicles Disrupts Nucleus Laminaris Development in the Chicken Auditory Brainstem

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Background: The pathway connecting nucleus magnocellularis (NM) to nucleus laminaris (NL) in the chicken auditory brainstem facilitates sound localization using interaural time differences. Projections from the cochlear (VIIIth) nerve innervate NM. Axons from NM neurons branch, projecting to the ventral contralateral dendrites of NL and the dorsal ipsilateral dendrites of NL. The cell bodies of NL form a thin monolayer of neurons surrounded by neuropil. We previously discovered that cleaved (active) caspase-3 is expressed in NM axons and NL dendrites prior to the period of programmed cell death. Caspase-3 is a proteolytic enzyme known for its roles in apoptosis. When caspase-3 is inhibited in ascending axon projections from NM, NL lamination is greatly impaired. To understand the mechanism through which caspase-3 regulates development, we first identified several hundred caspase-3 proteolytic substrates. GO term analysis revealed an abundance of substrates associated with exosomes/extracellular vesicles (EVs), suggesting a potential downstream modulator of caspase-3 activity during development.

Methods: If the primary substrates of caspase-3 modify EVs, then EVs may be necessary for circuit assembly downstream of caspase-3. If so, elimination of EVs would likely cause deficits in NL lamination, similar to those seen with caspase-3 inhibition. To test this possibility, we used DPTIP, a drug known to inhibit EV biogenesis. Fertilized chicken eggs were placed into ex-ovo culture on E2. We performed 2.5 μ M DPTIP and control injections at E8 and E9. After cryosectioning, Nissl-stained sections were imaged and NL cells were plotted. We used linear regression r-squared value of the plotted cell bodies to examine whether NL in DPTIP treated embryos was significantly less organized than control embryos.

Results: DPTIP treated animals showed irregular NL lamination compared to control animals (DPTIP $n = 8$, $r^2 = 0.797$; VCS $n = 9$, $r^2 = 0.955$; $p = 0.034$, Welch's Two Sample t-test). The cell bodies under normal conditions lie in a well-organized plane resulting in a linear array in sections. In contrast, DPTIP treated animals showed disrupted cell body ordering and significantly decreased linearity.

Conclusions: Our findings present the first evidence that extracellular vesicle inhibition disrupts NL lamination. This phenotypic similarity to caspase-3 inhibition is consistent with the idea that extracellular vesicles are required for proper development. This mechanism may represent a new model for the establishment of neural circuits.

SA81. Lineage Tracing of Repopulating Microglia in the Auditory Brainstem of Mice

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Background: Microglia, known for their immunological role in the central nervous system, have significant roles in central auditory circuit development. Auditory processing circuits, including those for computing sound source locations, require coordinated adaptations of precise developmental mechanisms. We previously showed that pharmacological depletion of microglia in mice from postnatal day P2 with a colony stimulating factor 1 receptor (CSF1R) inhibitor led to impaired synaptic pruning of the calyx of Held in the medial nucleus of the trapezoid body (MNTB), reduced ABR peak amplitudes, and increased ABR peak latencies. After withdrawal of the CSF1R inhibitor at P10, microglia returned gradually, beginning in lateral regions (including the ventral cochlear nucleus), later in middle regions (including the lateral superior olive), and beginning at about P22, the MNTB, which also mirrored the pattern seen during normal development. Significantly, this repopulation was associated with restored synaptic pruning and ABR measures, even at ages beyond the normal period of

circuit refinement. In contrast, long-term microglia depletion without repopulation led to retained polyinnervation and further decline in auditory function.

We hypothesized that repopulating microglia recapture developmental features of the original microglia during normal developmental stages. The goal of this project is to characterize the sources of these repopulating microglia and their developmental stages. Normally developing microglia arise from the yolk sac myeloid lineage and enter the brain early in development. Our preliminary differential gene expression results show that *Itgal*, a monocyte lineage gene, was upregulated in the repopulating microglia population of the CSF1R-inhibited mice relative to control mice. This observation suggests that, after microglia elimination, repopulating microglia could arise from peripheral monocytes.

Methods: We used a lineage tracing approach by injecting BLZ945, a CSF1R inhibitor, into mice every other day between P2 and P10, then observing microglia or monocyte markers to determine whether the new microglia have characteristics of peripheral monocytes. The *Ms4a3Cre-RosaTdT* reporter mouse shows recombination in granulocyte-monocyte progenitors (GMPs) and their progeny to express the TdTomato reporter. We immunolabeled repopulating microglia using the *Iba1* marker and evaluated the extent to which these repopulating microglia expressed the reporter, indicating a monocyte lineage.

Results: As expected, microglia were present throughout the auditory brainstem in control mice, but only in the lateral region in BLZ-treated mice at P21, consistent with our previous studies. In the selected area of the lateral auditory brainstem region of the *Ms4a3Cre-RosaTdT* mice, we found that up to 21% of *Iba1*-labeled repopulating microglia also expressed TdTomato, with up to 75% of the total monocytes overlapping with microglial *Iba1*. In contrast, we found almost no TdTomato-positive cells in the brainstems of control mice.

Conclusions: These findings suggest that a significant proportion of repopulating microglia arise from peripheral monocytes.

SA82. Muscarinic Modulation Activates Non-Selective Cation Channels and Increase the Excitability of Fusiform Cells of the Dorsal Cochlear Nucleus

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Background: The dorsal cochlear nucleus (DCN) of mammals plays a crucial role in suppression of self-generated sounds and monaural sound localization by integrating auditory and somatosensory information. Auditory information is conveyed by auditory nerve fibers, whereas somatosensory information is mediated by parallel fibers from granule cells in the cochlear nuclear complex. These inputs converge in fusiform cells, the glutamatergic principal cells in the DCN, which send their axons to the inferior colliculus. Therefore, fusiform cells are considered to play a central role in the DCN function. In the mammalian central nervous system, cholinergic signaling is essential for brain functions. The DCN receives cholinergic inputs from some brainstem regions, suggesting that these inputs modulate the output of fusiform cells to inferior colliculus. However, the direct evidence and underlying mechanisms of the cholinergic modulation in fusiform cells are still lacking.

Methods: In order to investigate the cholinergic modulation in fusiform cells, whole-cell patch-clamp recordings in acute slice preparations from the mouse brainstem (age: postnatal

17-27 days) were conducted. Several cholinergic agonists (Acetylcholine, nicotine, oxotremorine-M) were applied by bath-perfusion.

Results: Application of acetylcholine (100 μ M) and oxotremorine-M (a selective muscarinic agonist, 20 μ M) induced a clear inward current, while nicotine (10 μ M) did not elicit any significant response. This current had linear current-voltage relationship and the reversal potential of the current was approximately 0 mV. Additionally, this current was blocked by two types of TRP channel inhibitors, flufenamic acid (100 μ M) or 2-APB (100 μ M), indicating that the current was induced by the activation of non-selective cation channels with weak voltage dependence. Consistent with these findings, oxotremorine-M depolarized the membrane potential with reduction of membrane resistance, and increased both spontaneous and evoked firing in fusiform cells. Acetylcholine (500 μ M) had no significant effect on either excitatory and inhibitory synaptic transmission onto fusiform cells.

Conclusions: The muscarinic modulation enhances the excitability of fusiform cells by activating the non-selective cation channels, and may affect the integration of multimodal sensory inputs.

SA83. Central Auditory Synapse Alterations in Mice With Modified Medial Olivocochlear Efferent Activity

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Background: In many mammals, the auditory system is functionally immature at birth and undergoes postnatal refinement to achieve precise connectivity in adulthood. Spontaneous activity in the immature cochlea drives the organization of auditory brainstem circuits, a process modulated by the medial olivocochlear (MOC) efferent system. While the MOC pathway is known to protect against noise-induced hearing loss in adulthood, its developmental role remains less understood. We investigated how altered MOC activity during the critical period impacts the synaptic and morphological maturation of the calyx of Held (CH) synapse in the medial nucleus of the trapezoid body (MNTB)

Methods: We combined whole-cell patch-clamp recordings from MNTB principal neurons with 3D reconstructions from serial block-face electron microscopy (SBEM) at P12–14. Three mice genotypes were analyzed: wild type (WT), α 9KI (enhanced MOC activity), and α 9KO (absence of MOC activity). Morphological complexity of CH terminals was quantified using a custom Python pipeline based on graph theory, which enabled classification into distinct morphotypes.

Results: Electrophysiological recordings revealed that α 9KO mice showed electrophysiological responses comparable to WT. However α 9KI mice displayed reduced synaptic strength compared to WT, including smaller excitatory postsynaptic current (WT: 5.01 ± 1.58 nA, n=28; α 9KI: 3.61 ± 1.23 nA, n=25; α 9KO: 4.23 ± 1.34 nA, n=20; ANOVA: p=0.0013; Tukey WT- α 9KI, p=0.0008), stronger short-term depression during repetitive stimulation (100Hz: WT: $33.59 \pm 1.62\%$, n=12; α 9KI: $22.52 \pm 2.99\%$, n=10; α 9KO:

33.92±4.16%, n=11; ANOVA: p=0.0224; Tukey WT-α9KI: p=0.0403) and a decreased readily releasable pool size (WT: 374.02±29.11, n=10; α9KI: 253.01±21.78, n=12; α9KO: 323.21±21.39, n=9; ANOVA, p=0.0057, Tukey WT-α9KI, p=0.005). Morphological analysis indicated that α9KI animals exhibited a lower proportion of structurally complex CH terminals and evidence of loss in synaptic pruning, both in pre- and post-hearing stages. The α9KO mice displayed reduced morphological complexity but neither pruning nor electrophysiological performance were significantly altered (poly-innervation rate: WT/α9KO~10%; α9KI~30%).

Conclusions: Synaptic depression pattern in α9KI matches well with a higher proportion of simple CHs, according to literature linking structure and function at this synapse. Although previous studies in other auditory centers show both synaptic and morphological alterations when MOC efferent activity is lost, these results suggest that there may be some type of compensation at the level of the MNTB. Our findings demonstrate that enhanced MOC efferent activity during early development triggers profound modifications in both synaptic physiology and morphology at the CH–MNTB synapse. These results highlight the critical role of efferent pathways in shaping auditory brainstem maturation and suggest that altered efferent activity may contribute to long-term deficits in auditory processing.

SA84. The Function of NFI Transcription Factors in Cochlear Differentiation and Supporting Cell Plasticity

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Background: The loss of cochlear hair cells is a leading cause of hearing loss in humans. Research in mice shows that supporting cells can regenerate hair cells at early postnatal stages. However, the limited regenerative potential is lost as supporting cells undergo maturation. Our recent findings indicate that reprogramming cochlear supporting cells into progenitor-like cells improves their mitotic and hair cell-forming potential at more mature stages. The reprogramming of supporting cells into progenitor-like cells is accompanied by the downregulation of NFI transcription factors. NFI transcription factors control the differentiation of glial and neuronal cells in the developing brain and were recently identified as the main repressors of damage-induced cell proliferation in the mouse retina. The function of NFI transcription factors in cochlear supporting cell differentiation and supporting cell plasticity has yet to be addressed.

Methods: Published single cell RNA-seq data and NFIB-immunostaining was used to characterize cochlear Nfia, Nfib and Nfix expression. The Nfia, Nfib and Nfix genes were conditionally deleted in cochlear progenitors and supporting cells using Sox2-CreER transgenic Nfia,b,x triple floxed mice. For developmental experiments, tamoxifen was administered at E12, followed by EdU at E13.5 and EdU incorporation and the phenotype of control and Nfia/b/x deficient hair cells and supporting cells was analyzed at E18.5. For regenerative experiments, tamoxifen was administered at P3 and the mitotic and hair cell-forming potential of control and Nfia,b,x deficient supporting cells was analyzed at P5 using an organoid culture model.

Results: We found that Nfia and Nfib are co-expressed in cochlear progenitor cells, whereas Nfia, Nfib and Nfix genes are co-expressed in differentiating and maturing supporting cells. We found that loss of NFIA, B, and X function delayed the cell cycle exit and the differentiation of cochlear progenitor cells into hair cells and supporting cells. Furthermore, we observed defects in the medial-lateral pattern of hair cells, with more inner hair cells and

fewer outer hair cells being formed in Nfia/b/x triple knockout mice (TKO). Using a Tecta-cre strategy, we are currently examining whether loss of NFIA, B, and X during cochlear differentiation impacts hearing. Furthermore, we found that acute deletion of Nfia, Nfib, and Nfix increased the mitotic and hair cell-forming capacity of maturing cochlear supporting cells. We found that Nfia/b/x-deficient supporting cells formed larger organoids and proliferated at a higher rate than control. Moreover, we found that Nfia,b,x-deficient organoids formed small clusters of hair cells, whereas control organoids failed to do so.

Conclusions: Our research indicates that the transcription factors NFIA, NFIB, and NFIX promote cell cycle withdrawal and differentiation of cochlear progenitor cells. Additionally, our findings indicate that high NFIA, B, and X expression prevents cochlear supporting cells undergoing maturation from re-entering the cell cycle and from regenerating hair cells.

SA85. Loss of Zbtb20 Disrupts Sensory Epithelial Patterning and Maturation and Extends the Postnatal Hair Cell Regenerative Window in Mice

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Background: The transcription factor ZBTB20 is mutated in Primrose syndrome, a rare developmental disorder that can cause progressive hearing loss. A recent study (Xie et al. 2023 PNAS), using an early otic conditional knockout (cKO) mouse model of Zbtb20, reported profound hearing loss and atypical maturation of the non-sensory lateral cochlear epithelium. Here, we used cochlear and sensory epithelial-specific Emx2-cre and Tecta-cre lines to determine to what extent defects in the maturation of the cochlear sensory epithelium contribute to the hearing loss in Zbtb20 cKO mice. Furthermore, we use the chronic Emx2-cre Zbtb20 deletion model to determine whether blocking maturation of cochlear SCs enhances their mitotic and HC regenerative potential.

Methods: To study the role of ZBTB20 during cochlear development, Zbtb20f/f; Emx2-cre mice and control littermates were generated, and cochlear tissue was analyzed at Postnatal Day 0 (P0) through P30 using immunolabeling and histological techniques. Auditory Brainstem Responses (ABRs) were recorded at P30 to evaluate hearing capacity. Additionally, RNA sequencing (RNA-seq) was performed at P5 on isolated cochlear sensory epithelia. To evaluate the contribution of sensory epithelium defects to the reported hearing phenotype, Zbtb20f/f; Tecta-cre mice were generated. To profile regenerative capacity following chronic loss of Zbtb20, the Zbtb20f/f; Emx2-cre mice and control littermates were used to generate cochlear organoid and explant cultures at P5, which were then evaluated using microscopy, immunolabeling, EdU-labeling of proliferating cells, and RT-qPCR. In a subset of experiments the Pou4f3-DTR transgene was used to ablate HCs using diphtheria toxin.

Results: Deletion of Zbtb20 in the developing cochlear duct using Emx2-cre caused severe hearing loss. Notably, severe hearing loss was also observed in the Tecta-cre mouse model, which restricts the cKO domain to the sensory epithelium and Greater Epithelial Ridge (GER). Emx2-cre; Zbtb20 cKO mice exhibited patterning defects in the cochlear sensory epithelium, showing an increase in outer HCs (OHCs) and Deiter's cells. Notably, the apical tip showed no tapering of OHC rows, instead producing a blunt end with 4 rows of OHCs. RNA-seq and immunolabeling at P5 showed downregulation of key SC subtype-specific genes, such as Fabp7 in Hensen's Cells, and an upregulation of the stemness-related genes

Hmga2 and Sox11. Profiling of the Emx2cre; Zbtb20 cKO using a P5 organoid culture model showed increased organoid size and formation efficiency, increased proliferation, and increased HC regeneration.

Conclusions: This study identifies ZBTB20 as a master regulator of cochlear sensory epithelial differentiation and maturation. Loss of Zbtb20 in developing HCs and SCs leads to profound hearing loss, SC and HC patterning defects, and a delay in SC maturation. Furthermore, our findings indicate that halting cochlear maturation greatly enhances the mitotic and HC regenerative potential of cochlear SCs.

SA86. Chd7 and Sox2 Regulate Regional Organization of the Mammalian Cochlea and Expression of Vestibular Patterning Genes

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Background: Hereditary hearing loss and balance disorders are important clinical problems that have become easier to diagnose but have not greatly benefitted from advances in molecular therapies. An important step in designing therapies for hereditary hearing loss and balance disorders is understanding their underlying mechanisms. Chd7 encodes the ATP-dependent chromatin remodeler CHD7, mutated in CHARGE syndrome. Sox2 encodes Sex determining Region Y-box 2 family transcription factor SOX2. Our previous study showed that the expression of Chd7 and Sox2 is tightly regulated during inner ear development, and perturbation of the balance between these two genes leads to complex inner ear malformations, including shortened cochlea and malformed semicircular canals. In this study, we explored possible roles of Chd7 and Sox2 in regional organization of the mouse cochlea and expression of vestibular patterning genes.

Methods: Chd7 germline and conditional mutant (Chd7Gt/+; Chd7flox/flox), Sox2 inducible Cre knockin (Sox2CreER/+), and Pax2-Cre transgenic mice were used to study Chd7 and Sox2 in cochlear and vestibular development. E11.5 or E14.5 embryos were processed for in-situ analysis. Regional cochlear markers Fst, Efnb2 and Msx1 in the apex, and Inhba, Aldh1a3, and A2m in the base, were used. Expression of vestibular patterning genes Bmp4, Dlx5, Lmo4, and Otx1 was analyzed.

Results: In E14.5 double heterozygous (Chd7Gt/+;Sox2CreER/+) embryos, Fst, Efnb2 and Msx1 were highly expressed in the apical cochlea, similar to wild type littermates, and expression of cochlear basal markers, Inhba and Aldh1a3, was preserved in the base. However, A2m was greatly downregulated or absent in the basal cochleae of Chd7Gt/+;Sox2CreER/+ mutant embryos. Formation of the posterior vertical pouch, labeled by Dlx5 and Lmo4, was defective in E11.5 Chd7Gt/+;Sox2CreER/+ embryos. In contrast, formation of the anterior vertical pouch and the lateral wall (labeled by Lmo4 and Otx1) were normal in Chd7Gt/+;Sox2CreER/+ embryos.

Conclusions: These results demonstrate that the apical and basal cochlear identity was preserved in Chd7Gt/+;Sox2CreER/+ embryos. Downregulation of the basal marker, A2m, in Chd7Gt/+;Sox2CreER/+ embryos may reflect direct A2m expression regulation by Chd7. Chd7 and Sox2 cooperate to regulate expression of the vestibular patterning genes, which contributes to the semicircular canal defects in Chd7Gt/+;Sox2CreER/+ mice. These results will inform the design of novel molecular therapies to treat hearing and balance disorders associated with CHARGE syndrome.

SA87. Sox11 and Chd7 Act in the Same Gene Regulatory Network to Promote Inner Ear Development

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Background: Inner ear development requires precise spatiotemporal regulation of gene expression. Research has shown that two SRY-related high-mobility group box (SOX) transcription factors, Sox4 and Sox11, act as key regulators in inner ear morphogenesis. In the brain, SOX11 and SOX4 are essential neurogenic fate regulators and are direct targets of CHD7 (Chromodomain Helicase DNA binding protein 7). In humans, haploinsufficiency of CHD7 causes CHARGE syndrome which presents with inner ear dysplasia, hearing loss and balance impairment. Pathogenic variants in SOX11 are associated with Coffin-Siris syndrome and inner ear malformations. The specific mechanisms by which loss of CHD7 or SOX11 affects ear development are not fully understood but likely involve changes in downstream gene expression due to transcriptional regulation and nucleosome remodeling. In this study, we utilized mouse models to investigate whether Chd7, Sox4, and Sox11 operate within a shared or separate genetic regulatory network during inner ear development.

Methods: Embryonic inner ears with germline or conditional (Pax2Cre) deletion of Sox4, Sox11, and/or Chd7 were examined for morphological abnormalities using paint-filling and histological analysis. Molecular markers expressed during early inner ear development (E10.5-12.5) were assessed via qRT-PCR, immunofluorescence, and in situ hybridization. Laminin staining was assessed to visualize basement membrane integrity at the fusion plate. Cell Proliferation and apoptosis were evaluated using EdU incorporation and anti-Caspase3 assays, respectively.

Results: Severe abnormalities in the posterior and lateral semicircular canals were observed with loss of Sox11 or Chd7. In contrast, Pax2Cre;Sox4flox/flox inner ears exhibited no semicircular canal defects but displayed mild anomalies with enlarged common crus and endolymphatic duct. Sox11^{-/-} mutants exhibited delayed formation of the canal fusion plate, normal basement membrane laminin, reduced cell proliferation in the surrounding mesenchyme, and no aberrant cell death. Expression of Sox11 was reduced in Chd7 null ears, while expression of other genes known to regulate semicircular canal development (Otx1, Lmo4, Dlx5, and Bmp4) were normal in Sox11^{-/-} mutant ears.

Conclusions: Proper dosage of Sox4, Sox11, and Chd7 is crucial for normal development of vestibular structures in the mammalian inner ear. Sox11 functions downstream of Chd7 to regulate semicircular canal fusion plate formation and promote proliferation of cells in the surrounding otic mesenchyme.

SA88. Developmental Maturation of the Cochlear Sensory Epithelium Through Local Erk Signaling

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Background: Formation of the sensory epithelium in the cochlea is achieved through tight coordination of cell transformation and cell loss. The most conspicuous cellular changes in the developing cochlea occur medial to hair cells, as Kölliker's organ (greater epithelial ridge, GER) transforms into the inner sulcus. The columnar epithelial cells of the GER create the tectorial membrane and induce spontaneous activity of inner hair cells before hearing onset, functions that are essential for the normal development of the biomechanical properties of the cochlea and maturation of central auditory circuits. Previous studies have shown that changes in thyroid hormone cause the GER to regress prematurely or persist beyond the point of hearing onset, resulting in elevated ABR thresholds, morphological defects in the tectorial membrane, and changes in cochlear tuning. However, the cellular mechanisms that control the transformation of the GER are unknown. Here, we explored the contribution of ERK signaling to this process, a pathway that controls cellular patterning in epithelia throughout the body.

Methods: To visualize ERK signaling dynamics in the developing cochlea, we generated cochlear explants from CD1 mice expressing an ERK kinase translocation reporter (CAG-ERK-KTR-P2A-H2B-mRuby2 mice), and recorded changes in localization of mClover from the nucleus (unphosphorylated ERK) to the cytosol (phosphorylated ERK). To determine the functional role of ERK signaling we applied inhibitors of ERK and EGFR to cochlear explants and recorded changes in ERK signaling and cellular transformation.

Results: Longitudinal imaging in explants from pre-hearing mice revealed that ERK was activated sporadically in spatiotemporally restricted clusters of GER cells (abbreviated as Spatiotemporally Restricted ERK Signaling, "SpaRKS"). These events lasted for approximately 40 minutes, appeared throughout the GER, and were abolished following the addition of a MEK1 inhibitor. SpaRKS first appeared at the base of the cochlea at P5 and progressed from the base-to-apex, increasing in frequency along the length of the cochlear coil with age. Inhibition of EGFR and ERK caused apoptosis of GER cells, suggesting that SpaRKS regulate apoptosis of GER cells during development. Imaging cochlear explants in propidium iodide revealed that SpaRKS occurred around dying cells, suggesting that apoptosis leads to the release of EGFR ligands that temporarily promote the survival of surrounding cells within the GER. This mechanism provides a means to slow the rate of GER regression, allowing gradual cellular transformation to occur along the length of the cochlea.

Conclusions: Our studies indicate that local ERK signaling occurs within GER cells as it transforms into the inner sulcus. These clustered events are induced by the release of EGFR ligands from dying cells, promoting survival of neighboring cells. These SpaRKS allow the gradual formation of the inner sulcus, coordinating GER regression with the maturation of other aspects of the sensory epithelium at hearing onset.

SA89. PEA3 Transcription Factors Role in Cochlear Epithelial Development

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Background: The PEA3 subfamily of ETS transcription factors (ETV1, ETV4 and ETV5) contribute to the development of various tissues including nervous tissue, kidneys and lungs. They function through their evolutionary-conserved ETS domain that mediates DNA binding

and thereby regulates gene expression. In the cochlea, mesenchymal ETV4 and ETV5 operate downstream FGF signaling to regulate cochlear lengthening during early development. While epithelial FGF signaling has been shown to regulate multiple processes during cochlear development including placode induction, progenitor proliferation and differentiation, the role of potentially downstream PEA3 transcription factors has not been studied. In this work, we study the impact of epithelial-specific ETV1/4/5 single and combined deletions on cochlear epithelial development.

Methods: We generated single, double and triple mutant mouse models for the PEA3 family members through a conventional deletion of ETV4 and conditional deletions of ETV1 and ETV5 utilizing Sox2-driven inducible CreERT2. Mutant mouse embryos from pregnant females induced by tamoxifen at embryonic day (E) 12.5 and E13.5 were collected at E19.5 and analyzed using immunohistochemistry of whole mount tissues and cryosections. A One-Way ANOVA test followed by Tukey's multiple comparisons test were used to detect statistical significance and adjusted P value < 0.05 was considered significant. Single-cell RNA sequencing of cochlear duct cells from ETV1/4/5 triple mutant embryos and controls at E15.5 followed by 10x genomics cloud analysis were performed to analyze differentially expressed genes within the supporting cell cluster.

Results: ETV4/5 double and ETV1/4/5 triple mutants showed 22-35% increase in inner hair cell density throughout all cochlear turns with no change in outer hair cell density. TUJ1 staining showed disorganized innervation pattern to outer hair cells in both double and triple mutants. Hair cell densities and innervation in single mutant cochleae were comparable to controls. As for supporting cells, ETV4/5 double mutants showed 30-40% reduction in NGFR+ cell density and 5-33% increase in CD44+ cell density without any change in SOX2+ cell density. ETV1/4/5 triple mutants showed a more severe phenotype including 85-93% reduction in NGFR+ cell density and 64-78% increase in CD44+ cell density indicating a possible dose-dependent role of the PEA3 subfamily members in pillar cell development. Sox2-CRE positive control cochleae were comparable to Sox2-CRE negative cochleae and displayed no change in hair cell or supporting cell densities. Single-cell RNA sequencing of ETV1/4/5 triple mutant embryos at E15.5 showed 26 upregulated genes within the Fgfr3+ supporting cell cluster (Negative Binomial Exact Test, Log2 Fold change > 1, adjusted P value < 0.05) including 6 genes involved in glutamatergic synapses. Only 2 genes were downregulated when compared to triple heterozygote controls.

Conclusions: PEA3 family of transcription factors regulate epithelial development in mammalian cochlea. PEA3 deletion causes upregulation of glutamatergic synapse-specific genes within the supporting population. Pathways downstream PEA3 factors are currently under investigation.

SA90. Working Towards Human iPSC-Derived Vestibular-Cochlear Assembloids

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Background: The human inner ear comprises two interconnected systems: the cochlea and the vestibular system. While recent advances have enabled the generation of cochlear and vestibular organoids from human induced pluripotent stem cells (iPSCs), these systems are

typically developed in isolation, missing the anatomical unity seen in vivo. This separation poses limitations, especially when studying drug distribution or modeling diseases that affect the entire inner ear.

Methods: To address this, we propose a novel in vitro strategy to assemble a unified inner ear model by fusing separately differentiated vestibular and cochlear organoids. Organoids are initially directed toward vestibular or cochlear fate until day 22 (d22), corresponding to the stage when otic vesicle formation and lineage specification occur. At this point, we merge the organoids using different approaches, including direct co-culture in the same dish and a 3D-printed structure to push fusion of the two organoids.

Results: To distinguish and track the developmental contributions of each organoid, we used a combination of a wild-type iPSC line and a SOX2+ reporter iPSC line. After 4 days in the same well, the vestibular and cochlear organoid fused together. These "assembloids" are cultured until day 90 (d90), when the majority of vestibular and cochlear cell types have matured. The tissues are then fixed and analyzed via immunohistochemistry to assess cellular composition, organization, and potential interactions, and compared to individually cultured vestibular and cochlear organoids.

Conclusions: As a next step, we aim to apply this inner ear assembloid platform to model genetic hearing and balance disorders. Using iPSCs derived from a patient with a mutation causing USH2A disease, we generate disease-specific cochlear-vestibular assembloids. Furthermore, we test the therapeutic potential of previously established antisense oligonucleotides to counteract the disease phenotype within this integrated system.

SA91. ZMYM4 May Mediate Some Aspects of Spiral Ganglion Neuron Development and Specification

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Background: Zinc finger MYM-containing type 4 (ZMYM4) has recently been shown in *Xenopus laevis* to be expressed within the pre-placodal epithelium (PPE) and the neural crest cells (NCCs) during early embryogenesis. Loss of ZMYM4 results in a down regulation of NCC and PPE markers with a concomitant expansion of the neural ectoderm while overexpression had little to no effect. These data, combined with recent studies into other members of the ZMYM family, suggest that ZMYM4 may be acting as a pioneer factor during early specification of the PPE and NCCs. As the PPE gives rise to the otic placode and later, the sensorineural epithelium of the inner ear, we hypothesize that ZMYM4 may be involved in cell fate specification within the inner ear. More specifically, as it has been linked to neurodevelopment and the neural epithelium expands with loss of ZMYM4 at the expense of the NCCs and PPE, it is likely that ZMYM4 may play a role in the specification of the neural components of the inner ear, the spiral ganglia neurons (SGNs).

Methods: Whole mount and sectioned mouse embryos from embryonic day (E) 10.5 through to adulthood were analyzed to establish a temporospatial timeline of ZMYM4 expression throughout ontogeny. We then collected SGNs at E14.5 and performed scATACseq and CUT and RUN to establish whether ZMYM4 functions as a pioneer factor during neuronal subtype specification.

Results: ZMYM4 is expressed within the sensorineural epithelium from the earliest stages examined and is maintained in the population of cells that delaminate from the sensorineural

epithelium to form the SGNs. By E14.5, ZMYM4 has become restricted to the nucleus of all SGNs. As this corresponds to the stage at which SGNs initiate subtype specification, it is possible that ZMYM4 functions as a pioneer factor during SGN maturation. This nuclear expression of ZMYM4 is then maintained throughout ontogeny. As more reports of patients with congenital neurodevelopmental disorders are presenting with variants in ZMYM proteins, there is strong evidentiary support for its function as a regulator of neuronal maturation. We are currently analyzing scATACseq and CUT and RUN data to determine both whether ZMYM4 is bound to DNA and its potential downstream targets.

Conclusions: These data present only the very first investigation into the putative functions of ZMYM4 during inner ear development. In the future, we plan to investigate the function of ZMYM4 during SGN development and specification via both shRNA-mediated knockdown and overexpression within the ear. Overall, ZMYM4 is an exciting avenue of future research and may represent some of the uninvestigated epigenetic landscape of sensorineural development within the ear.

SA92. Itga8 Deletion From Supporting Cell Disrupts Cytoskeletal Microtubule Stabilization: Possible Role of YAP and ERM Signaling

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Background: Supporting cells in the cochlea provide structural support that is critical for hair cell function. However, it is not well understood how their interactions with the extracellular matrix connect to the organization of their internal cytoskeleton. We examined how loss of integrin $\alpha 8$ (Itga8) in supporting cells affects YAP signaling and ERM protein function, two key regulators of cytoskeletal stability and cell mechanics.

Methods: We used conditional knockout mice (Sox2-Cre $\times$ Itga8^{fl/fl}) to delete Itga8 specifically in supporting cells. We then assessed changes in cell adhesion, cytoskeletal organization, and YAP and ERM signaling using RNA scope and immunostaining approaches.

Results: Loss of Itga8 disrupted junctions in pillar and Deiter's cells, weakening their mechanical connection to hair cells. RNA-scope data showed that Itga8 deletion altered YAP levels, shifting supporting cells toward reduced mechanosensitivity and premature cell cycle exit. From postnatal day 10 to 60, mutants showed progressive breakdown of actin and microtubule structures. In particular, pillar cells lost their characteristic acetylated microtubule bundles, which were nearly absent by P60. Changes in ERM protein levels further impaired their ability to connect actin and microtubules. We also noticed the age dependent hearing loss in mutant mice.

Conclusions: Our study identifies Itga8 as a key regulator linking YAP-driven gene programs with ERM-mediated cytoskeletal stability in supporting cells. This mechanosensing pathway offers a potential therapeutic target for hearing loss, as supporting cell dysfunction can precede and contribute to hair cell degeneration.

SA93. Role of Integrin $\alpha 8$ in Spiral Ganglion Neuron Development

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Background: Integrin $\alpha 8$ (Itga8) is a bi-directional adhesion receptor involved in cytoskeletal signaling, cilia biogenesis, and neuronal development. Although its function in sensory hair cells has been partially described, its role in auditory connectivity particularly in spiral ganglion neurons (SGNs), which relay sound information from the cochlea to the brain remains poorly understood.

Methods: We examined Itga8 expression in SGNs using RNAscope and immunohistochemistry across developmental stages up until after the onset of hearing, which have not been reported before. To assess function, we generated conditional knockouts (Celf4-iCreT2:Itga8^{fl/fl}) and evaluated neuronal morphology with confocal imaging, and quantitative analyses via Python based pipeline. Presynaptic integrity was assessed by CtBP2 synaptic counts, while auditory function was examined using auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE) testing.

Results: Itga8 expression persisted in SGNs from birth through adulthood, suggesting a sustained role during auditory maturation. Conditional deletion of Itga8 in SGNs led to significant reductions in presynaptic ribbon counts in both inner and outer hair cells, decreased neurofilament density, and disorganized or misoriented neuronal fibers along the cochlear spiral. Quantitative analysis confirmed reduced neurite integrity in knockout mice. We are currently analyzing our ABR and DPOAE assessments to further elucidate on Itga8 function in auditory circuit.

Conclusions: These findings identify Itga8 as a critical regulator of SGN development and synaptic homeostasis. Loss of Itga8 disrupts neuronal morphology, connectivity, and auditory processing, underscoring its importance in cochlear circuit integrity.

SA94. Lipid-Regulating Drug Fenofibrate as a Candidate Therapy for Aminoglycoside-Induced Hearing Loss in Mice

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Background: Aminoglycoside antibiotics remain indispensable for treating severe infections, yet their clinical utility is limited by a high incidence of permanent sensorineural hearing loss. Despite decades of research, no FDA-approved pharmacological agents currently exist to prevent aminoglycoside-induced hearing loss (AGIHL). The pathogenesis of AGIHL is multifactorial, involving oxidative stress, mitochondrial dysfunction, synaptic degeneration, and metabolic dysregulation, highlighting the need for therapeutic approaches that target multiple mechanisms. Fenofibrate, a peroxisome proliferator-activated receptor alpha (PPAR α) agonist widely prescribed for dyslipidemia, has found to be a lead compound through screening against kanamycin-induced hair cell loss in zebra fish. It has been reported to exert antioxidant and anti-inflammatory effects in extra-auditory tissues, raising the possibility of repurposing this well-characterized drug for otoprotection. However, its bioavailability in the cochlea, efficacy against AGIHL, and impact on inner ear metabolism remain poorly understood.

Methods: Six-to-seven-week-old C57BL/6 corrected for cdh23 mutation were co-treated with 600mg/kg of kanamycin twice a day and fenofibrate 20mg/kg/day for 14 consecutive days. ABRs and DPOAEs were recorded before and at 30 days post kanamycin and fenofibrate co-treatment. Mice were then sacrificed, and cochleae isolated for the analyses of IHC, OHC, and synapses. To avoid the saturation effect, mice were treated for 10 consecutive days and sacrificed after 24 hours of the last treatment, cochlear lysate were prepared to

isolate lipids and polar lipids were analysed using LC/MS, Mid-modiolar sections were prepared to perform RNA scop to analyse PPAR alpha, Acox1, Acs14, ApoE, TNF, IL-6 and MHC1 transcripts. Pharmacokinetic was performed after one-time oral dose of fenofibrate.

Results: Fenofibrate reached peak serum levels of 120 ng/ml and cochlear levels of 380 ng/ml at 3 hours, with prolonged retention in the cochlea at 24 hours. Kanamycin exposure induced ~30 dB SPL ABR threshold shifts, reduced peak-1 amplitudes, and synaptic ribbon loss. Co-treatment with fenofibrate significantly attenuated threshold shifts (10–15 dB SPL), preserved peak-1 amplitudes, and maintained synaptic ribbons across most frequencies, except at 16 kHz. Lipidomic profiling identified 307 species across 12 classes. Kanamycin disrupted cochlear lipid subclasses (ePC, PS, ePS, PA, LPE) without altering systemic levels. Fenofibrate normalized elevated ePC (38:4) and further reduced LPEs, partially restoring lipid balance. Although polar lipids were not fully recovered, these changes correlated with improved hearing sensitivity. RNAscope analysis showed that PPAR α , inflammatory genes (TNF, IL-6, MHC1), and lipid-regulating enzymes (Acox1, Acs14, ApoE) were dysregulated by kanamycin but restored to baseline with fenofibrate co-treatment.

Conclusions: Collectively, these results provide preliminary evidence supporting fenofibrate as a candidate for repurposing to prevent AGIHL. Furthermore, the observed modulation of lipid metabolic pathways highlights a novel mechanism of drug action that may contribute to long-term auditory protection.

SA95. Live Imaging of Presynaptic Ribbons in the Noise-Exposed Juvenile Mouse Cochlea

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Background: Moderate acoustic exposure leads to cochlear synaptopathy, characterized by the loss of ribbon synapses between inner hair cells (IHCs) and spiral ganglion neurons (SGNs). This form of damage manifests as impaired sound processing despite preserved hearing thresholds. Unlike permanent threshold shifts caused by hair cell death, synaptopathy can be reversible. While live imaging of zebrafish neuromasts have provided key insights into ribbon formation, trafficking, and turnover, they do not reproduce physiologically relevant noise damage. Development of a robust model for direct visualization of synaptic ribbons in the noise-exposed mammalian cochlea is necessary to better understand how noise-induced cochlear synaptopathy develops and can be treated

Methods: To directly observe synaptic changes after noise trauma, we developed an ex vivo model using cochlear explants from 7-week-old RIBEYE-tagRFP mice, in which presynaptic ribbons are genetically labelled with tagRFP. Explants were imaged using a Nikon A1R upright confocal microscope with a 60 $\times$ water immersion objective and stage-top incubation. Mice were exposed to 2-hr, 98 dB SPL 8-16 kHz broadband noise, a stimulus known to induce synaptopathy in vivo. Animals were euthanized at multiple timepoints after completion of the noise exposure, and cochlear explants were dissected and imaged within 10 minutes of euthanasia. Confocal z-stacks of the basolateral poles of inner-hair-cells at the 8-10-kHz region of the cochlea were acquired for up to 30 minutes, and presynaptic ribbons were tracked for dynamic changes over time in number, size, and subcellular location.

Results: This imaging protocol enabled detection of ribbon motion with 3-dimensional subcellular spatial and ~1-minute temporal resolution, such that ribbon movement could be clearly tracked during the 30-minute recording periods. Simultaneous imaging with FM1-43

live dye confirmed overall inner-hair-cell morphological stability without evidence of cell swelling, blebbing, or death. Following noise exposure, presynaptic ribbons on aggregate showed increased motility. Subsets of ribbons demonstrated distinct behaviors, including unanchoring from paired synapses along the cell periphery, directed cytoplasmic transport, and punctal fusion. These events unfolded dynamically over the hours to days following exposure.

Conclusions: This live imaging model reveals that presynaptic ribbons undergo specific significant rearrangements in response to acoustic trauma. The observed motility suggests a window of synaptic plasticity during which targeted interventions may stabilize or restore vulnerable contacts. This system provides a powerful platform for studying synapse recovery mechanisms and testing therapeutic strategies for noise-induced hearing deficits.

SA96. Sec14-Like3, a Lipid Transporter Protein, Attenuates the Cochlear Damage Caused by Intense Noise Exposure

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Background: The cochlea is a crucial organ in sound reception. The cochlear sensory epithelium, along with the outer cochlear wall, plays a vital role in effectively transmitting auditory signals via a delicate mechanism. Recent studies have highlighted the significant role of lipids in intracellular signal transduction within the cochlear sensory epithelium^{1,2}. However, the detailed mechanisms underlying this process remain poorly understood.

To investigate the involvement of lipids in sound reception, we focused on the lipid transporter protein Sec14-like3, which has been implicated in lipid transport in the alveolar and bronchial epithelium³.

Methods: Wild-type and knockout mice with C57/BL6 background obtained from the Department of Biochemistry and Molecular Biology (Nippon Medical School, Tokyo, Japan) were used in this study.

Sec14l3/l4 double-KO mice were created using the CRISPR-Cas9 system by designing sgRNAs in exon5 each of Sec14l3 and Sec14l4. The sequence surrounding exon5 of Sec14l3 is inserted into exon5 of Sec14l4. This insertion causes a frameshift in both Sec14l3 and Sec14l4. Therefore, we decided to use these mice as Sec14l3/l4 double KO mice. ABR measurement and immunohistochemistry for 8-OHdG and 4-HNE were performed. Statistical analyses were conducted using IBM SPSS Statistics version 29.0.2. The data for ABR thresholds, hair cell counts and relative expression (8-OHdG and 4HNE) were compared by unpaired two-tailed Student's t-test.

Results: Immunohistochemical images showing the expression of Sec14l3 (green) protein in the organ of Corti of WT and KO mice. Immunohistochemical images showing the expression of Sec14l4 (green) protein in the organ of Corti of WT and KO mice. Scale bar is 5 μ m. Immunohistochemical images showing the expression of Sec14l4 (green) protein in the spiral ganglion, spiral ligament, and stria vascularis of WT mice. Scale bar is 20 μ m. Cell nuclei are stained blue, and hair cells are stained red. WT: wild type group; KO: knock out group. OHC: outer hair cell; IHC: inner hair cell.

Sec14l3 expression was observed in the organ of Corti (Deiters' cells and inner pillar cells) No clear Sec14l4 expression was observed in the cochlea. Under normal conditions, no

significant differences in auditory brainstem response (ABR) thresholds were detected among the groups. However, following noise exposure, significant differences in the ABR threshold shifts were observed in each group. Across all frequencies, the ABR threshold shifts in KO mice were greater than those in WT mice.

Conclusions: we designed sgRNAs in exon5 of each Sec14l3/14 and created Sec14l3/14 double-knockout (KO) mice using the CRISPR-Cas9 system. Wild-type (WT) and Sec14l3/14 double KO mice exhibited no differences in hearing at steady state. However, significant hearing loss was observed in Sec14l3/14 double KO mice following acoustic exposure. These results suggested that Sec14l3 plays an important role in sound reception.

SA97. Role of Rabs in Regulating Aminoglycoside Ototoxicity in Sensory Hair Cells

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Background: Aminoglycoside antibiotics are used clinically to treat serious bacterial infections. Unfortunately, they can result in hearing loss, tinnitus and balance disorders. The aminoglycosides enter the hair cells of the inner ear via mechanoelectrical transducer channels in the sensory hair bundle and/or via endocytosis. Both routes of entry eventually lead to delivery of the aminoglycosides to the lysosome with slower delivery causing an increase in toxicity, possibly due to increased impact on other cytosolic structures (e.g., ribosomes). However, the molecular mechanisms whereby ototoxic antibiotics are trafficked through the cells are poorly understood. Intracellular trafficking is heavily dependent on vesicle transport events which are in turn controlled by Rab proteins. This family of vesicle-transport proteins has been well studied, particularly in cell assays, and their association with known cellular pathways and effectors is relatively well described, particularly for those Rabs described as either essential or associated with human disorders. We sought to identify which RabGTPases are associated with intracellular trafficking of the aminoglycosides and therefore which pathway may be involved.

Methods: 5-day post fertilisation (dpf) pou4f3:GFP zebrafish larvae were treated with either 10 μ M gentamicin or control medium for 3 hrs, a time point before any overt hair-cell death occurs. The larvae were then dissociated using trypsin/EDTA and hair cells isolated using fluorescent cell sorting with a FACSMelody Cell Sorter and RNA extracted. Three control and three gentamicin matched samples were sent for QuantSeq. Sequences were analysed using the Galaxy platform and EdgeR. 5dpf AB zebrafish larvae were treated with either 10 μ M gentamicin or control medium for 3 hrs, anaesthetised, fixed in 4% PFA overnight and dehydrated in methanol. RNAscope was performed using probes for a hair cell marker (myo6b) and either rab44 or rab1aa. Larvae were mounted in ProLong Gold onto slides with glass shims and imaged using a Zeiss LSM980 confocal. Images were analysed using ImageJ.

Results: RNAseq data has identified a number of rabGTPases that are differentially expressed in the gentamicin-treated hair cells. Those highly upregulated include rab44 shown to have a role in early endosomes and lysosomal exocytosis, and rab1aa which is involved in endoplasmic reticulum (ER) to Golgi trafficking and autophagy. Expression analysis using RNAscope shows an increase in the total area of rab1aa positive puncta in lateral-line hair cells of larvae treated with gentamicin compared to control larvae.

Conclusions: These data suggest roles for rab1aa and rab44 in the transport of aminoglycosides inside the hair cells. To confirm we are producing rab44 knock-out and

dominant-negative rab1aa knock-in zebrafish to assess how altering Rab-mediated transport affects the survival of aminoglycoside-treated hair cells.

SA98. Activation of Aryl Hydrocarbon Receptor Signaling Rescues Cochlear Hair Cells From Damage

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Background: Preventing cochlear hair cell damage caused by ototoxins and acoustic trauma remains a major challenge in the auditory field, with limited therapeutic interventions available. Identifying genes and signaling pathways that mediate protective or reparative responses to oxidative stress and injury is critical. The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor known to regulate xenobiotic metabolism and cellular homeostasis, but its role in the cochlea and in repair following ototoxic injury remains unexplored. Using a cochlear explant model, we previously found that pharmacological activation of AhR with an endogenous ligand 24 hours after neomycin exposure significantly increased outer hair cell survival in the middle and basal turns compared to controls treated with neomycin only. In this study, we investigated the expression pattern of AhR in the cochlea under physiological and damaged conditions, as well as mechanisms downstream of AhR involving anti-inflammatory and anti-oxidative pathways.

Methods: AhR expression in the cochlea was assessed using the knockin AhRtdTomato mouse model, where tdTomato expression is driven by endogenous AhR regulatory elements. Cochlear whole-mount microdissections and mid-modiolar sections were examined at key postnatal stages. To model injury, cochlear explants from C57BL/6 mice were treated with 600 μ M neomycin for 24 hours, followed by pharmacological activation with the AhR agonist Oto-39. Activation of the AhR target gene, CYP1A1, and the impact on inflammatory responses was assessed using quantitative RT-PCR.

Results: Constitutive AhR expression was observed in supporting cells, Schwann cells, and glial cells surrounding spiral ganglion neurons, with low basal expression in the organ of Corti. Following neomycin treatment, AhR expression was increased in supporting cells specifically in the region medial and lateral to hair cells compared to controls treated with media only. Pharmacological activation of AhR with Oto-39 increased CYP1A1 expression, confirming activation of AhR signaling in the cochlea. Preliminary results further revealed reduced expression of the pro-inflammatory cytokine TNF- α after AhR activation. Studies to further elucidate the contribution of AhR signaling to antioxidant responses (via the AhR–Nrf2 pathway) are ongoing.

Conclusions: Our findings demonstrate that AhR is constitutively expressed in cochlear supporting cells and is upregulated following ototoxic injury. Activation of AhR signaling reduces pro-inflammatory responses and enhances hair cell survival, suggesting a novel reparative role for AhR in the cochlea.

SA99. NADPH Oxidase 3 Inhibition Preserves Hearing in Mice After Stereotactic Radiosurgery

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Background: Stereotactic radiosurgery (SRS) is a standard treatment for vestibular schwannoma but carries a substantial risk of progressive and irreversible hearing loss. NADPH oxidase 3 (NOX3), an enzyme predominantly expressed in the inner ear, has been identified as a key source of oxidative stress driving cochlear injury. We investigated whether genetic deletion or transient inhibition of Nox3 using small interfering RNA (siRNA) could preserve auditory function and cochlear structures following SRS.

Methods: C57BL/6J mice (6 weeks old) received unilateral 32 Gy cochlear irradiation in two experimental arms. In the genotype arm, littermates included wild-type (Nox3^{+/+}; n=8), heterozygous (Nox3^{+/-}; n=8), and Nox3-deficient (Nox3^{-/-}; n=8) mice. In the siRNA arm, wild-type mice were randomly assigned to posterior semicircular canal injection of either siRNA targeting Nox3 (siNox3; n=14) or scrambled control (siScr; n=14) 48 hours before SRS. Irradiation was delivered using the Gamma Knife ICON system with cone-beam CT co-registered to MRI and CT-based mouse atlases for precise unilateral cochlear targeting. Auditory brainstem responses (ABR) were obtained at baseline, 1, and 4 weeks post-SRS. At week 4, cochleae were collected for histological analysis. Statistical analyses employed a two-way repeated measures ANOVA with Bonferroni correction.

Results: Following 32 Gy unilateral SRS, wild-type mice developed progressive high-frequency hearing loss by week 4, whereas Nox3-deficient littermates showed complete preservation of auditory thresholds, with significant differences between groups across 2.8–32 kHz ($p < 0.01$ to $p < 0.0001$). Heterozygous mice exhibited an intermediate phenotype. Contralateral ears showed no ABR threshold shifts in any group. Under suprathreshold stimulation, wave I and III amplitudes were significantly lower in wild-type than in Nox3-deficient mice after click stimulation (both $p=0.02$) and at 32 kHz ($p=0.002$ and $p=0.03$). Histology revealed preserved inner hair cells in all groups. Wild-type mice showed marked outer hair cell (OHC) and ribbon synapse loss with mild spiral ganglion neuron (SGN) reduction at the basal turn, partially attenuated in heterozygous and absent in Nox3-deficient mice.

Similarly, siNox3-treated mice were largely protected from SRS-induced hearing loss, with significant differences in auditory thresholds compared with the siScr-treated group across 5.6–32 kHz ($p < 0.05$ to $p < 0.0001$). Under suprathreshold stimulation, wave I and III amplitudes at 32 kHz were significantly lower in siScr compared with siNox3-treated mice ($p=0.03$ and $p=0.04$). Histology in siScr-treated mice resembled that of wild-type animals. In contrast, siNox3-treated mice showed preserved ribbons and SGNs, with only mild OHC loss at high frequencies.

Conclusions: Genetic deletion and siRNA-mediated knockdown of Nox3 preserved auditory function and prevented cochlear injury following SRS, establishing NOX3-derived oxidative stress as a key mediator of ototoxicity. Given the predictable timing of SRS for vestibular schwannoma in clinical practice, a NOX3-targeted intervention represents a feasible and selective strategy to prevent hearing loss without compromising tumor treatment.

SA100. Effects of Military Service on Auditory Brainstem Response Morphology

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Background: Military Veterans often report hearing concerns despite normal or near-normal audiometric thresholds, which suggest relatively normal outer hair cell function. However, many Veterans experienced significant degrees of loud noise exposure during their military service that may result in neural changes to the auditory system. Efforts to detect neural changes due to noise exposure using the auditory brainstem response (ABR) have produced mixed results. These inconsistencies may reflect the limits of conventional ABR analyses, which typically rely on visual inspection of the ABR to extract features such as peak latencies and amplitudes. While these features are clinically useful, they capture only a small portion of the ABR waveform and may overlook broader morphological patterns. Our automated modeling approach, the Gaussian Mixture Model Feature Extraction Technique (GMM-FET), quantifies a wider set of waveform features and may provide a more sensitive means of identifying cochlear deafferentation and other damage to the central auditory nervous system.

Methods: ABR waveforms generated in response to high intensity tone-bursts at multiple frequencies were collected from 330 adults (95 Veterans and 235 non-Veterans) with audiometric thresholds ≤ 40 dB HL at Boys Town National Research Hospital (BTNRH), Utah State University (USU), and the VA National Center for Rehabilitative Auditory Research (NCRAR). ABR waveforms will be analyzed by both visual inspection and the GMM-FET, which extracts up to 73 features per waveform. Logistic regression will be used to predict Veteran status from the ABR waveform features, controlling for potential outer hair cell damage as measured by standard and high-frequency audiometric thresholds and otoacoustic emissions. The BTNRH and USU datasets will be used to train, reduce features, and cross-validate the logistic regression model, while the NCRAR dataset will be reserved to test the model performance.

Results: Preliminary results using the BTNRH dataset show that prediction of Veteran status using visually extracted ABR waveform features is no better than chance, while GMM-FET extracted features perform significantly better than chance. Important GMM-FET extracted features of the ABR that differ significantly between Veterans and non-Veterans include latency and amplitude, but also area and curvature changes to waves II through IV. The addition of the USU data and testing the model on the NCRAR dataset will evaluate the generalizability of these findings.

Conclusions: Veterans and non-Veterans exhibit clear differences in ABR waveform morphology using our automated feature extraction technique that are not clear using traditional visual inspection. These diffuse changes to the ABR beyond wave I, are not explained by audiometric thresholds or otoacoustic emissions, suggesting the presence of subclinical neural changes associated with military service that may extend beyond outer hair cell loss and cochlear deafferentation from noise exposure.

SA101. Mitochondrial Protection by Oncomodulin: A Key Mechanism in Preventing Noise-Induced Hearing Loss

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Background: Noise-induced hearing loss (NIHL) is a major cause of sensory impairment worldwide, commonly linked to mitochondrial dysfunction and hair cell death within the cochlea. Together, these conditions compromise auditory function and survival of sensory cells. Mitochondria play a central role in maintaining cellular energy metabolism and calcium homeostasis, and their dysfunction often triggers oxidative stress and cell death. Previous studies show that oncomodulin (OCM), a calcium-binding protein, uniquely enriched in cochlear outer hair cells (OHC), modulates intracellular calcium signaling and is important for maintaining auditory function. However, its specific role in mitochondrial stability and protection against cellular stress-induced damage is not understood.

Methods: To investigate the role of OCM in mitochondrial homeostasis, we have employed both in vitro and in vivo approaches. Recent studies in our lab show that OCM knockout (KO) mice had significantly elevated auditory brainstem response (ABR) thresholds following noise exposure (120 dB SPL, 2 hrs) compared to wild-type (WT) controls. This functional decline was accompanied by reduced expression of the mitochondrial marker TOM20, suggesting impaired mitochondrial maintenance. In this study, we used HEI-OC1 cells, which are derived from auditory cells as an in vitro model to investigate cellular and sub-cellular modifications during induced stress. These cells were transfected with OCM or control vectors and exposed to thapsigargin, an endoplasmic reticulum stressor, or hydrogen peroxide, an oxidative stress inducer. Cellular and subcellular responses were assessed using Mitosox live cell imaging for superoxide detection, mitochondrial membrane potential assays, immunofluorescence for mitochondrial markers and cytochrome C release to detect apoptosis.

Results: After transfection with OCM, HEI-OC1 cells showed marked resistance to thapsigargin-induced stress. Specifically, OCM-transfected cells displayed reduced mitochondrial superoxide accumulation, preservation of mitochondrial membrane potential, and maintenance of TOM20 expression at near-normal levels. Furthermore, OCM expression was associated with reduced cytochrome C release, indicating suppression of mitochondrial-mediated apoptotic signaling. Collectively, these findings demonstrate that OCM enhances mitochondrial resilience and limits oxidative injury under stress conditions.

Conclusions: Our study provides compelling evidence that OCM serves a protective role in maintaining mitochondrial homeostasis within cochlear hair cells. By buffering calcium and stabilizing mitochondrial function, we hypothesize that OCM reduces oxidative stress and prevents initiation of apoptosis, thereby preserving OHC viability and auditory function. These findings highlight OCM as a critical factor in reducing susceptibility to NIHL and suggest that strategies to enhance or mimic OCM activity may represent a novel therapeutic avenue for mitigating hearing loss associated with acoustic trauma.

SA102. Repurposing Pravastatin and Metformin for the Treatment and Prevention of Noise-Induced Hidden Hearing Loss in a Mouse Model

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Background: Noise-induced hidden hearing loss (NIHHL) is a subtle form of hearing damage in which thresholds recover while synapses between inner hair cells and afferent fibers are permanently damaged. Currently, there are no FDA-approved therapeutics. Using a noise-response molecular atlas and DrugCentral, we identified statins and metformin as

potential NIHL therapeutics. Both target the mevalonate pathway, upregulated in key auditory cells after acoustic trauma. Here we report the completed study evaluating whether these drugs protect against NIHL.

Methods: Seventy-two male and seventy-two female B6CBAF1/J mice were used. Pravastatin (25 mg/kg/day) and metformin (200 mg/kg/day), alone and in combination, were delivered in drinking water beginning at 8 weeks. Baseline ABR and DPOAE thresholds were recorded at 9 weeks. At 10 weeks, mice were exposed to noise (97 dB SPL, 8–16 kHz, 2 h) to induce NIHL without a permanent threshold shift. ABR and DPOAE were measured at 24 hours, 1 week, and 6 weeks post-exposure. Cochlear tissue was collected for histology (OHC loss; IHC synapse counts with CtBP2/GluR2). ABR and DPOAE thresholds were assessed using two-way ANOVA with Tukey's multiple comparisons, and wave-I amplitudes with two-way ANOVA followed by Dunnett's test versus placebo; adjusted p values are reported. Data were analyzed with $\alpha = 0.05$.

Results: Consistent with a hidden hearing loss-inducing exposure, thresholds in all groups returned to baseline by one week post-noise and remained stable at six weeks. The main outcome, ABR threshold shifts at 24 hours post-exposure, showed robust protection in male mice treated with either metformin alone (16 kHz, $p=0.0009$; 24 kHz, $p < 0.0001$; 32 kHz, $p < 0.0001$) or the combination of metformin and pravastatin (16 kHz, $p=0.0009$; 24 kHz, $p < 0.0001$; 32 kHz, $p < 0.0001$). In females, both pravastatin (16 kHz, $p=0.012$; 24 kHz, $p=0.0045$; 32 kHz, $p=0.0005$) and the combination (16 kHz, $p=0.0002$; 24 kHz, $p < 0.0001$; 32 kHz, $p=0.0007$) significantly reduced ABR threshold shifts, while metformin alone was not protective.

The second key outcome, ABR wave-I amplitude at one week post-exposure, showed significantly larger responses in males treated with combination therapy compared to placebo at 32 kHz, mid-level intensities ($p < 0.05$), consistent with partial preservation of auditory nerve function.

Supporting measures from DPOAE analysis showed additional protective effects: in males, the combination improved responses; in females, pravastatin and the combination reduced threshold shifts. Histological analysis is ongoing and will evaluate OHC loss and IHC synaptic integrity to further define protective mechanisms.

Conclusions: After completion with an expanded sample size across sexes, our data demonstrate that metformin plus pravastatin improves early auditory recovery after acoustic trauma. Effects were frequency- and sex-dependent, with combination therapy providing the most consistent protection in males and pravastatin contributing in females. Ongoing histology will clarify synaptic preservation underlying these outcomes and will be presented at February 2026 meeting.

SA103. Electroencephalographic Measurements of Auditory Development in the First Year of Life

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Background: Phonetic features rely partly on temporal acoustic changes in speech, such as frequency modulation (FM) and amplitude modulation (AM). However, many of the neural mechanisms supporting temporal processing are immature at birth due to the protracted development of the auditory system. This study aims to explore auditory development in the first year of life by using electroencephalographic (EEG) methods. Three recordings targeting

different auditory structures, from the auditory nerve up to the auditory cortex are measured in infants using a longitudinal design.

Methods: We recruited a group of 25 3-month-old infants who are following-up at 10-month-olds. We also recruited a group of 31 adults for comparisons. All listeners were awake throughout the EEG recordings and were presented with AM-sound to record AMFR (amplitude modulation following response) and FM stimuli to record FFR (frequency following response). The AMFR was recorded for a 4-min-long pure-tone sinusoidally modulated at 8 Hz (carrier frequency = 1027 Hz) presented via loudspeakers (at 75 dB SPL). The FFR was recorded for a saw-tooth wave sound centered at 220 Hz and presenting FM fluctuations of 4 Hz. This sound was presented via loudspeakers (at 60 dB SPL) in a continuously manner for 4 min with first positive polarities and then another 4 min with negative polarities. We also recorded the wave V of ABRs (Auditory Brainstem Responses) for all listeners.

Results: Wave-V ABRs were consistently observed in all infants while they were awake. We however observed a faster transmission of the sound signal in adults than in infants. For the FFR, adults showed a higher response of neural encoding response to FM than 3- and 10-month-old infants. However, for the AMFR, no difference in amplitude of the neural response to AM was observed with age.

Conclusions: Our results are promising for the clinical assessment of auditory functions in infants. ABR results suggest that the neural transition of sound from the ear to the brainstem is not yet fully developed by the age of 10 months. FFR results suggest that neural phase locking for sounds with FM variations of 4 Hz is not adultlike at 10 months of age, while the neural encoding of slow AM (8 Hz) is by 3 months. Therefore, this study provides information about auditory development in the first year of life from the early to late stages of neural sound processing.

SA104. Targeted AAV Gene Therapy for Genetic Hearing Loss in Pendred Syndrome and DFNB4

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Background: Mutations in the SLC26A4 gene are a leading cause of inherited hearing loss, accounting for over 6% of cases. These mutations result in Pendred syndrome and DFNB4, two forms of sensorineural hearing loss for which there are currently no effective treatments beyond supportive care like hearing aids or cochlear implants.

Methods: We have designed an AAV-SLC26A4 vector capable of improving the hearing thresholds and preventing the inner ear pathology in mouse models with SLC26A4 mutations that recapitulate aspects of the human disorder. Human SLC26A4 coding sequence was packaged in the AAV-S capsid, which shows high transduction in native SLC26A4-expressing cells in neonatal mouse and adult NHP cochlea. Vectors were injected into postnatal day 1 (P1) mouse and adult NHP cochlea via the round window membrane (RWM).

Results: To understand the vector transduction efficiency and expression specificity, wild-type (WT) mice were injected with an AAVS-SLC26A4 HA-tagged vector. After ~30 days, cochleas were processed for histology. Cochlear cells natively expressing Slc26a4 produced the HA-tagged SLC26A4 protein, demonstrating cell-specific vector expression.

We tested the vector's efficacy in two different mouse models of SLC26A4-related hearing loss.

(1) Severe SLC26a4 Knockout (KO) Model: Mice with severe-to-profound hearing loss showed a dose-dependent improvement in hearing, with some mice experiencing a remarkable 30-40 dB improvement. This treatment also preserved outer hair cells (OHCs), a key structure for hearing, which were otherwise lost in the untreated mice.

(2) Variable L236P Knock-in Model: In a second model representing a common human mutation, the treatment resulted in a statistically significant improvement of 20-30 dB in median hearing thresholds. Even in animals with high hearing thresholds, OHC loss was significantly reduced by approximately 50%, highlighting the protective effect of the therapy on cochlear anatomy.

Finally, to assess potential efficacy in humans, the AAVS-SLC26A4-HA vector was injected through the RWM of cochleas of cynomolgus monkeys (*M. fascicularis*). As in mouse, HA labeling demonstrated cell-specific expression.

Conclusions: The novel AAVS-SLC26A4 vector successfully and specifically expressed the human SLC26A4 protein in the correct cochlear cells of both mice and NHPs. It demonstrated significant therapeutic effects in two different mouse models of the disease, leading to improved hearing and preservation of critical inner ear structures. These results suggest that AAVS-SLC26A4 is a promising candidate for further development as a gene therapy for SLC26A4-related hearing loss in humans.

SA105. Improvement of Hearing and Prevention of Inner Ear Pathology in a Severe SLC26a4 Mouse Model of Pendred Syndrome/DFNB4

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Background: Mutations in the SLC26A4 gene, encoding pendrin protein, are responsible for Pendred syndrome and DFNB4, two autosomal recessive disorders causing sensorineural hearing loss. Pendrin is essential in the inner ear for regulating endolymphatic pH and maintaining the endocochlear potential. Without functional pendrin inner ear development is severely disrupted, leading to profound deafness. We developed a novel AAVS-SLC26A4 gene therapy to restore SLC26A4 function by directly delivering the functional gene to the ear.

Methods: Human SLC26A4 coding sequence was packaged in the AAV-S capsid, which shows high transduction in native SLC26A4-expressing cells in neonatal mouse and adult NHP cochlea. We used an in-vitro activity assay to ensure that the AAVS-SLC26A4 vector generates functional protein. To test in vivo efficacy AAVS-SLC26A4, we injected the vector into the round window membrane of postnatal day 1 (P1) *Slc26a4* knockout mice that exhibit severe-to-profound hearing loss. Animals were tested by auditory brainstem response (ABR) ~30 days after injection.

Results: To measure the activity of the pendrin protein, we developed an assay using a halide-sensitive YFP mutant in HEK293 Phoenix Eco cells. We measured baseline YFP fluorescence in a high chloride solution, then added a high iodide solution to quantify the quenching caused by pendrin's iodide exchange activity. YFP fluorescence was quenched in a

dose-dependent manner based on the amount of AAVS-SLC26A4 delivered. Cells that didn't receive AAVS-SLC26A4 showed no significant quenching, confirming that the fluorescence reduction was directly caused by the gene therapy. Furthermore, we confirmed that pendrin expression correlated with the observed YFP quenching.

To test the hearing efficacy of AAVS-SLC26A4 *in vivo*, we injected Slc26a4-KO mice with various doses of the AAVS-SLC26A4 vector expressing human SLC26A4 and show that AAVS-SLC26A4 dose-dependently improved hearing thresholds with some animals achieving 30-40 dB improvement compared to the best hearing uninjected KO animal. We then explored whether a vector using the mouse Slc26a4 gene, instead of the human one, might be even more effective. Preliminary findings suggest this is the case, particularly at lower frequencies.

To assess the effect of the treatment on cochlear anatomy, we quantified outer hair cell (OHC) numbers in SLC26a4-KO animals. Untreated Slc26a4-KO mice showed loss of OHCs whereas treated mice had a striking preservation of OHCs with 100% normal OHCs in most animals, making them histologically indistinguishable from the wild-type controls.

Conclusions: We demonstrate that the novel AAVS-SLC26A4 gene therapy vector is highly effective in producing functional pendrin protein. When tested in a severe mouse model of Slc26a4-related hearing loss, the vector showed targeted expression of the human gene and significantly improved hearing thresholds and prevented the loss of OHCs. These findings represent a major step toward a potential clinical treatment for SLC26A4-related hearing loss.

SA106. Preclinical Genome Editing Therapy Safely and Efficiently Restores Progressive Hearing Loss in Kcnq4 G285S Mouse

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Background: Autosomal dominant hearing loss is typically progressive and postlingual, creating a potential window for intervention. Mutations in KCNQ4 gene cause DFNA2A, one of the most common forms that represent ~10% of all DFNA cases. KCNQ4 encodes a voltage-gated potassium channel essential for outer hair cell (OHC) function, and pathogenic mutations exert a dominant-negative effect, abolishing potassium currents and causing OHC degeneration. Standard AAV-mediated gene replacement therapy is unsuitable for dominant mutations, highlighting the unmet need for precision genome editing approaches. A mouse model with the equivalent human mutation Kcnq4 G285S/+ showed a delayed onset and progressive hearing loss, making it an ideal model to develop a gene editing therapy to rescue hearing for DFNA2.

Methods: We used a genome editing strategy by designing and screening gRNAs for diverse CRISPR systems, including SpCas9, SaCas9-KKH, and the adenine base editor SpCas9-NG-ABE8.0e, to specifically target the Kcnq4 G285S mutation. By PiggyBac transposon insertion, we created NIH-3T3 cells and human HEK293 cells engineered to carry the KCNQ4 G285S mutation. Editing outcomes were assessed using next-generation sequencing (NGS). The sgRNA and SaCas9-KKH were packaged into a single AAV2 for injection into adult Kcnq4 G285S/+ inner ear. Auditory function was assessed by ABR and DPOAE.

Results: In vitro screening identified multiple gRNAs for SpCas9 with robust on target editing, but also with modest editing on the WT Kcnq4 allele, leading to the exclusion of the

SpCas9 gRNAs. In contrast, multiple SaCas9-KKH gRNAs exhibited efficient editing targeting the KCNQ4 G285S allele, with undetectable editing in the KCNQ4 WT allele. We constructed AAV2-SaCas9-KKH/sgRNA-Kcnq4 and injected it into 4-week-old Kcnq4G285S/+ inner ear, with the uninjected ears as controls. 12 weeks after the injection, we detected lower ABR thresholds in injected compared to the uninjected control ears, an indication of hearing rescue. Lower DPOAE thresholds were also observed in the injected ears. To assess long-term auditory function by genome editing, hearing tests were conducted at 24 weeks after injection. Significantly lower ABR and DPOAE thresholds were detected in injected ears compared to the uninjected control ears. Greater reductions in ABR and DPOAE thresholds were detected at 24 weeks than at 12 weeks post injection, an indication of long term rescue.

Conclusions: Our study provides proof of concept that genome editing can safely and efficiently restore auditory function in Kcnq4-related dominant hearing loss. By allele-specific disruption of the KCNQ4 G285S mutation, we demonstrate genome editing as a promising therapeutic strategy for DFNA2A and other dominant forms of genetic deafness. AAV2-mediated delivery of SaCas9-KKH/sgRNA-Kcnq4 in an adult DFNA2 mouse model resulted in significant hearing rescue, laying the groundwork for an ongoing IND-enabling study to move the work toward the clinic.

SA107. AAV-Mediated Gene Therapy to Target Vestibular Schwannoma

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Background: Vestibular schwannomas (VSs) represent the most common cerebellopontine angle tumors and typically present with progressive sensorineural hearing loss, tinnitus, and vertigo. Sporadic VSs arise unilaterally, while the genetic condition neurofibromatosis type II (NF2) is typically associated with bilateral VSs. There are no FDA-approved medications specifically for VS or NF2. However, recent preclinical studies and promising trials involving otoferlin gene therapy have suggested that adeno-associated virus (AAV)-based gene replacement therapy may be a viable treatment option.

Methods: Human sporadic and NF2-associated VSs were collected intraoperatively. Expression of AAV receptor (AAVR) was analyzed in VS tissue cryosections using immunofluorescent staining. Results were confirmed with single-cell RNA-seq data. Primary cultures were established and 9 different serotypes/variants (AAV2/1, AAV2/2, AAV2/6.2, AAV2/8, AAV2/9, AAV2/Anc80L65, AAV9-PhP.B, AAV9-PhP.eB, AAV2.7m8) were assessed for their transduction efficiency. Over 4 days, live cell imaging with Incucyte enabled precise visualization of eGFP expression and p75 (CD271) positivity to quantify Schwann cell transduction. The effects of preincubation of human VS primary cultures with anti-AAVR antibody before the addition of AAVs was analyzed. The supernatant of transduced primary human VS cell cultures was examined via ProcartaPlex to determine inflammatory reactions upon exposure to various AAV serotypes.

Results: AAVR is highly expressed in human Schwann cells. AAV2/2, AAV2/6.2, and AAV2.7m8 were identified as the most promising serotypes in terms of eGFP mean intensity and cell-specific double-fluorescent quantification (p75 and eGFP). The above-mentioned

AAVR blocking experiment demonstrated a reduced transduction of both, a natural (AAV2/6.2) and a synthetic AAV (AAV2.7m8). The ProcartaPlex data showed upregulation of several markers with IL-6 being the highest, but statistically significant differences were not observed between the AAV serotypes.

Conclusions: This study demonstrates the potential of AAV-mediated gene therapy to effectively target vestibular schwannoma and should accelerate the development of translational therapeutic interventions to treat this disabling condition.

SA108. A Gjb2:mCherry Reporter Mouse Reveals Spatial and Temporal Expression Patterns of Gjb2

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Background: GJB2, mutated in the most common human hereditary deafness, is expressed by diverse populations of epithelial cells and fibrocytes in the mammalian cochlea. A successful gene therapy requires expression of a GJB2 transgene in the same cells and at the same time as endogenous expression. Here, we developed a novel Gjb2 reporter mouse to investigate expression during key developmental stages.

Methods: We generated a Gjb2:mCherry reporter mouse by CRISPR-mediated knock-in of donor DNA encoding the mCherry coding sequence, under control of the endogenous Gjb2 promoter and preceding the Gjb2 coding sequence. Three stop codons inserted downstream of mCherry allowed reporter expression but disrupted Gjb2 expression from that allele. Homozygous Gjb2:mCherry/mCherry mice were embryonic lethal, as expected, so we used heterozygous Gjb2:mCherry/+ mice. Auditory thresholds were measured at P30 to determine whether reporter knock-in impaired hearing. We assessed reporter expression with native mCherry fluorescence or with anti-mCherry antibodies. To assess proper targeting of a therapeutic vector, Gjb2:mCherry cochleas were injected at P1 with an AAV9-PHP.B vector encoding wild-type GJB2 with a C-terminal HA tag. Cochleas were harvested at P10 for comparison of viral Gjb2-HA and mCherry expression.

Results: mCherry fluorescence revealed that reporter knock-in was successful. ABR and DPOAE results showed no statistically significant threshold difference between the Gjb2:mCherry/+ mice and Gjb2:+/+ littermate controls. In frozen sections and whole mounts, we observed mCherry expression at P2, P8, P14 and P30. To evaluate the efficacy of viral gene delivery, Gjb2:mCherry/+ mice were injected at P1 with an AAV9-PHP.B vector encoding HA-tagged GJB2. Cochleas harvested at P10 and immunolabeled for the HA tag showed that viral GJB2.HA expression localized to the same cell populations as the mCherry reporter.

Conclusions: Our novel Gjb2:mCherry mouse model provides a powerful tool for visualizing Gjb2 expression over time and confirms that AAV-mediated Gjb2 delivery can target the correct cell populations within the cochlea. This Gjb2:mCherry/+ mouse model can be used as a platform to assess vector efficiency, specificity, and toxicity for the development of therapeutic treatments for GJB2-related hearing loss.

SA109. Inner Hair Cell Synaptic Remodeling Following Otof Gene Therapy

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Background: Inner hair cell (IHC) ribbon synapses form the first synaptic relay of the auditory pathway. The presynaptic protein otoferlin is essential for calcium-dependent vesicle release, and mutations in the OTOF gene cause DFNB9 auditory neuropathy. Otoferlin deficiency disrupts synapse maturation, resulting in fewer hypertrophic synapses and profound deafness. Dual-AAV-Otof gene therapy reinstates glutamatergic release and the hearing function. Here, we addressed whether this functional rescue occurs with, or without, restoration of normal synaptic architecture.

Methods: Deaf5 mice carrying a homozygous missense mutation in Otof affecting Ca²⁺-binding and abolishing IHC synaptic transmission were injected at 6 weeks of age with 2 μ L of dual-AAV1-Myo15-myc-mOtof, while uninjected Deaf5 littermates served as untreated controls. Age-matched wild-type mice (WT) were included as normal-hearing control. Analyses were performed at 13 weeks of age. Following auditory brainstem response (ABR) recording and operant behavioral testing, cochleae were immunolabeled for CtBP2 (presynaptic ribbons), Homer1 (postsynaptic densities, PSDs), and a Myc tag (fused to Otof transgene) to verify transgene expression. Samples were then imaged with confocal microscopy, and quantitative as well as morphometric analyses of ribbons and synapses were performed using Imaris-based segmentation.

Results: Uninjected otoferlin-deficient Deaf5 mice were profoundly deaf, as demonstrated by both absent ABR and behavioral responses. Quantitative analysis revealed a ~50% reduction in synapse number per IHC compared to WT, along with hypertrophic synaptic elements: presynaptic ribbon volumes (re: WT) were increased by ~27% in untreated and ~23% in AAV-treated mice, while postsynaptic density volumes were increased by ~53% in untreated and ~9% in AAV-treated mice. Delivery of dual-AAV-Otof restored sound-evoked behavioral responses and ABR thresholds to near-WT levels. However, in treated mice, IHCs contained no more synapses than untreated Deaf5, and ABR wave I amplitudes remained reduced, showing a ~55% decrease at 80 dB SPL for 8 kHz stimulation compared to WT. These data demonstrate that re-establishment of IHC–auditory nerve fiber (ANF) glutamatergic transmission restores hearing thresholds and partially normalizes synaptic element size but does not recover synapse number.

Conclusions: Otoferlin deficiency in Deaf5 mice causes a reduction of IHC–ANF synapses, rendering the remaining synapses functionally silent. Dual-AAV-Otof therapy restores auditory thresholds but not the synapse count, with synaptic morphology only partially restored. Future serial 3D-EM analysis of synapse anatomy in untreated and AAV-treated mice will help assess the relationship between structural rescue and functional recovery, providing essential insight into the durability of synaptic repair and plasticity, and guiding studies on how treatment age, aging, and noise exposure influence the long-term success of gene therapy.

SA110. AAV-Mediated Specific Gjb2 Gene Therapy Restores Hearing in DFNB1 Mouse Models

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Background: DFNB1 represents the most prevalent form of hereditary non-syndromic hearing loss to date, accounting for 20% to 50% of all cases. Pathogenic mutations in the Gjb2 gene, which encodes connexin 26, constitute the leading cause of autosomal recessive hereditary deafness. Although adeno-associated virus (AAV)-mediated gene therapy has shown promise in partially restoring auditory function, its application for treating Gjb2-related deficiencies remains constrained by the limited specificity and low efficiency of existing AAV vectors.

Methods: We evaluated multiple AAV serotypes and identified that co-administration of AAV1 and AAV-ie effectively targeted Gjb2-expressing cells in vivo. Furthermore, the SCpro promoter was utilized to drive transgene expression, thereby enhancing specificity and eliminating ectopic expression-induced ototoxicity.

Results: Co-administration of AAV1 and AAV-ie, each carrying the exogenous Gjb2 gene under the control of the SCpro promoter, achieved precise and efficient gene delivery. This combined AAV system successfully restored auditory function in Gjb2 conditional-deficient mouse models, with hearing thresholds improving to a best level of 40–45 dB SPL.

Immunofluorescence analysis confirmed robust recovery of GJB2 protein expression in the cochlea of both models. Moreover, the dual-AAV system mediated efficient transduction in the cochleae of Bama miniature pigs. When administered into the inner ear of cynomolgus monkeys, it induced no auditory impairment or systemic toxicity, supporting its favorable efficacy and safety profile in large animal models.

Conclusions: This study introduces a targeted gene therapy strategy for Gjb2-related hearing loss using a dual-AAV system and a specific promoter to avoid ototoxicity while restoring hearing in DFNB1 mouse models. The approach demonstrated strong efficacy and a favorable safety profile in large animals, supporting its clinical translation. Further studies are needed to optimize long-term stability in humans. Overall, this strategy provides a precise and safe treatment for the most common form of genetic hearing loss, offering promising therapeutic potential for this currently incurable condition.

SA111. Determining Tropism of AAV Serotypes in the Neonatal Mouse Cochlea

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Background: Over 170 genetic mutations have been identified to cause hearing loss, with many amenable to replacement via gene therapy in preclinical models. More recently, clinical trials in gene therapy have revealed promising results for otoferlin-related hearing loss.

Adeno-associated viruses (AAVs) have become increasingly popular as a vehicle to deliver gene replacement for genetic hearing loss because of their high tropism and low immunoreactivity. Currently, there are multiple AAVs reported with different tropisms when injected through the posterior semicircular canal (PSCC). We set out to characterize the

tropism of 5 AAVs in neonatal mice after PSCC injection, with the goal of determining one that is most efficient in gene therapy for Tmprss3 KO mice.

Methods: C57Bl/6 postnatal 3-day-old (P3) mice were injected with 1 ul of each virus at 5 nl/s through PSCC injections. 5 different viruses were tested: AAV2/8-CMV.EGFP ($5.32\text{E}+13$ GC/mL), AAV2/9-CMV.EGFP ($7.55\text{E}+12$ GC/mL), AAV2/Anc80L65.CMV.EGFP ($1.89\text{E}+13$ GC/mL), AAV2.7m8-CAG-GFP ($5\text{E}+12$ GC/mL), AAV-ieK558R-CAG-mCherry-3xFLAG -WPRE ($1.33\text{E}+13$ GC/mL). Each virus was mixed with 1:25 trypan blue (1:25 v:v) to facilitate visualization during injection. Mice were sacrificed 7 days after injection, and immunohistochemistry of whole mounts was performed to determine expression of mCherry or GFP using an anti-GFP antibody. Tissues were also immunostained for inner and outer hair cells (IHCs and OHCs, Myo7a) and support cells (SCs, Sox2). Cell counts of hair cells and support cells were performed in each cochlear turn in whole mounts to compare the transduction efficacy of the different AAVs. Saline injection and the contralateral ear were used as controls.

Results: For AAV2/8-CMV.EGFP and AAV2/9-CMV.EGFP, IHC transduction was most efficient in the apical turn ($60.4\pm7.5\%$ and $61.4\pm10.4\%$, respectively) with a decreasing gradient towards the basal turn ($35.9\pm2.6\%$; $17.6\pm3.0\%$). AAV2/8-CMV.EGFP had overall higher transduction of the IHCs, OHCs, and SCs ($46.0\pm4.3\%$, $7.2\pm2.7\%$, $11.7\pm2.4\%$) compared to AAV2/9-CMV.EGFP ($31.2\pm4.3\%$, $6.8\pm3.7\%$ and $5.6\pm4.0\%$). While AAV2/8-CMV.EGFP had minimal transduction efficacy in the contralateral, uninjected ear only in the basal turn ($2.5\pm0.7\%$), AAV2/9-CMV.EGFP had no transduction in the contralateral, uninjected ear. There was minimal toxicity of the AAVs with little to no degeneration of the native IHCs, OHCs, or SCs after injection. Ongoing experiments aim to characterize the transduction efficacy of AAV2/Anc80L65.CMV.EGFP, AAV2.7m8-CAG-GFP, and AAV-ieK558R-CAG-mCherry-3xFLAG -WPRE.

Conclusions: There are significant differences in the transduction efficacy of different AAVs in the IHCs, OHCs, and SCs. The results of this study can guide selection of AAV to deliver gene replacement for Tmprss3 mutant mice.

SA112. Honokiol Attenuates Cisplatin-Induced Hearing Loss in Sirtuin 1–Deficient Mice

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Background: Sirtuins are composed of an NAD⁺-dependent deacetylase family expressed in different cellular compartments which are critical for oxidative stress response. Sirtuin 1 (SIRT1) is one of the cytoplasmic sirtuin, and its roles in hearing loss and protection are studied with contradictory results. We found that SIRT1 is activated by honokiol, a known SIRT3 activator which showed hearing protective effects in different hearing impairments. In this study, the roles of SIRT1 in hearing protection are studied using cisplatin-induced hearing loss as the stressor. Since Sirt1 knockout is embryonically lethal, Sirt1 insufficient (Sirt1^{+/-}) mice are used in this study, combined with gene therapy mediated by adeno-associated virus 2 (AAV2) tagged with green fluorescent protein through posterior semicircular canal (PSCC) injection.

Methods: Wild-type (Sirt1^{+/+}) and Sirt1^{+/-} mice were assigned to four different experimental groups for cisplatin and honokiol treatment: control, honokiol (10 mg/kg),

cisplatin (4 mg/kg), honokiol + cisplatin. Honokiol was given for four consecutive days following auditory testing at 8, 10, and 12 weeks of age through intraperitoneal (i.p.) injection (4 doses/cycle for 3 cycles). Cisplatin was given only one hour after the first dose of honokiol on each cycle (1 dose/cycle for 3 cycles), through intravenous (i.v.) injection. Hearing was assessed by auditory brainstem response (ABR) measurement, in a frequency range of 8–36 kHz. In addition, three more groups will be assigned for gene therapy: AAV2-vector, AAV2-SIRT1 untreated, and AAV2-SIRT1 cisplatin-treated. Viral transfection will be performed on Sirt1^{+/-} mice through PSCC injection at the age of 6 weeks. Same cisplatin treatment protocol and ABR recording procedure will be followed. Immunostaining is used for verifying SIRT1 expression and hair cell loss. Biochemical assays of SOD2 activity and reactive oxygen species will be performed to verify SIRT1 activity. RT-PCR will be used to verify the involvement of SIRT1 downstream genes. Light sheet microscopy will be used to verify the AAV2 transfection profile in the intact cochlea.

Results: SIRT1 insufficiency does not significantly affect the hearing of the animals under normal conditions, as the ABR threshold did not change between the wild-type and Sirt1^{+/-} mice in the control groups. Honokiol alone does not affect the hearing of the animals, either. However, with the stress of cisplatin, Sirt1^{+/-} mice showed significantly elevated ABR threshold at most frequencies after the first cycle (10 weeks), which was not observed in wild-type animals. The average threshold elevation, compared to the wild types, was 20.6 dB. Honokiol pre-treatment alleviated this cisplatin-induced ABR threshold shifts by 19.6 dB. No animal loss due to cisplatin treatment has been observed so far.

Conclusions: SIRT1 deficiency exacerbates cisplatin-induced hearing loss. Honokiol gives protection in Sirt1^{+/-} mice, either through activating SIRT1, other sirtuins, or other mechanisms, which will be further investigated in future gene therapy and RT-PCR.

SA113. Functional Significance of Sirtuin 3 in the Protection Against Cisplatin-Induced Hearing Loss

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Background: Sirtuin 3 (SIRT3) is a mitochondrial deacetylase involved in redox maintenance, and its role in hearing protection has gained increasing attention in recent years. We previously reported early-onset hearing loss in male Sirt3 knockout (Sirt3^{-/-}) mice, which was alleviated by honokiol, a known SIRT3 activator. It is unclear how the absence of SIRT3 affects vulnerability to hearing stress such as cisplatin ototoxicity, and whether honokiol can still provide protection. In addition, the molecular pathways underlying honokiol's protective effects via SIRT3 are not well defined. These questions are addressed in this study.

Methods: Male wild-type (Sirt3^{+/+}) and knockout (Sirt3^{-/-}) mice were assigned to experimental groups: vehicle, honokiol (10 mg/kg), cisplatin (4 mg/kg), or honokiol + cisplatin. Treatments were given for four consecutive days following auditory testing at 6, 8, and 10 weeks of age. Honokiol was injected intraperitoneally one hour before cisplatin. Hearing was assessed by auditory brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAE) at 8–36 kHz. Survival was monitored. Immunostaining for SIRT3 expression and cochlear damage, biochemical assays of SOD2 activity and reactive oxygen species, and RNA-seq of outer hair cells are in progress.

Results: Cisplatin significantly elevated ABR and DPOAE thresholds, with more severe threshold shifts in Sirt3^{-/-} mice, both in onset time and frequency range. At the end of the 3rd cycle (week 12), cisplatin treatment induced a threshold elevation of 17 dB and 40 dB at 32 kHz, in wild-type and Sirt3^{-/-} mice, respectively. In honokiol pre-treated groups, this threshold shift is reduced to 5 dB and 23 dB, respectively. DPOAE thresholds showed a similar pattern, with more severe threshold shifts in Sirt3^{-/-} mice. Survival analysis through day 84 showed 76% survival in wild-type cisplatin-treated mice and 55% in Sirt3^{-/-}. With honokiol pre-treatment, survival was 71% in wild-type and 75% in Sirt3^{-/-} mice. Kaplan–Meier analysis confirmed significant differences with cisplatin exposure (log-rank $\chi^2 = 12.4$, df = 3, p = 0.006) but not with honokiol pre-treatment.

Conclusions: Honokiol attenuates cisplatin-induced hearing loss in both wild-type and Sirt3 knockout mice, suggesting that protective pathways beyond SIRT3 contribute to this effect. Ongoing RNA-seq of outer hair cells, combined with molecular and imaging assays, will help define the mechanisms by which SIRT3 and honokiol attenuate cisplatin-induced hearing loss.

SA114. Intracochlear Drug Delivery Mitigates Cochlear Implant Trauma and Promotes Preservation of Synapses

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Background: Cochlear implantation (CI) is a highly effective treatment for individuals with severe to profound sensorineural hearing loss. However, insertion of the electrode array can cause electrode insertion trauma (EIT) resulting in damage to sensory cells, which can lead to the loss of residual hearing. Targeted pharmaceutical interventions aimed at these pathological pathways hold significant promise for preserving residual hearing after cochlear implantation. Through a preclinical rat model, we aim to evaluate the potentials of hearing preservation of a novel drug eluting cochlear implant electrode by quantification of the preservation of sensory hair cell populations, synaptic integrity, and neurite architecture as well as potentials of cellular proliferation and regenerative processes using high-resolution immunohistochemistry and confocal microscopy.

Methods: Sprague-Dawley rats (6-8 weeks old) were divided into three groups: nonimplanted (control group), implanted with a standard non-drug eluting electrode (EIT group), and implanted with a drug R eluting electrode (Drug group). CI Electrode administration was performed intraoperatively via cochleostomy. Cochlear dissections were performed for each group at day 30 post CI. The cochlea was then decalcified, and the Organ of Corti (OOC) was dissected into three cochlear turns: Base, Middle, Apex. OOC sections were then subjected to immunostaining using DAPI, FITC, CtBP2 antibody, and Ki67 followed by labelling with Alexa Fluor-conjugated secondary antibodies. Confocal microscopy was used to generate high-resolution z-stacks of the OOC while ImageJ software was used to conduct hair cell quantification. Auditory Brainstem Response (ABR) testing was performed pre-operatively and at endpoint to measure auditory threshold shifts across frequencies.

Results: Hearing thresholds determined through ABR testing demonstrated significantly decreased threshold shifts in the Drug group compared to the EIT group. Through confocal

imaging and analysis of viable hair cell percentages, Drug R significantly improved the preservation of sensory hair cells compared to the EIT group. Drug R additionally induced neurite formation and promotion of cochlear synapses as well as proliferation of Ki67 positive cells within the cochlea demonstrated by way of confocal imaging.

Conclusions: Localized intracochlear delivery of Drug R via drug-eluting electrodes demonstrates potential to attenuate electrode insertion-induced pathophysiological cascades, including synaptopathy, and neurite degeneration. Preservation of sensory hair cells and promotion of neurite outgrowth may enhance electrode–neural interface integrity, potentially improving speech perception outcomes in cochlear implant recipients. This targeted pharmacological strategy addresses a critical unmet clinical need for preserving residual hearing. Future investigations will focus on longitudinal evaluation of neuroprotective effects in chronic implantation models and pharmacokinetic and pharmacodynamic optimization for sustained therapeutic delivery.

SA115. Neuro- And Neurite-Protective Benefits of Proprietary Kv7.4-Activating Otoprotective Drug Candidates Through Mitochondrial Modulation

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Background: Kv7.4 potassium channel activators are considered good candidate otoprotective drugs with additional potential for auditory function enhancement due to the strong expression of Kv7.4 channels in the plasma membranes of cochlear outer sensory hair cells (Rim et al., 2021; Peixoto Pinheiro et al., 2022). However, recent reports have also suggested a significant cardio- and neuroprotective potential of Kv7.4 channel activation in the inner membranes of mitochondria (Testai et al., 2016; Paventi et al., 2022). We here report that our proprietary Kv7.4 activator drug candidate ACOU082, under development for the enhancement and preservation of natural hearing in chronic/progressive disorders such as age-related hearing loss, exhibits significant capacity for neuro- and neurite protection through direct action on mitochondria.

Methods: Primary cultures of dissociated rat cortical neurons from E15 Wistar rat pups were cultured under standard conditions in 96 well plates. On DIV13, after 1h of pre-treatment with control/test compounds, excitotoxic injury was induced with glutamate (20 μ M) in presence of control/test compound for 20 min. Subsequently glutamate was washed out and fresh culture medium with control/test compounds added for additional 24 hours. On DIV14, cultures were fixed with 4% paraformaldehyde in PBS, permeabilized using PBS containing 0.1% saponin and 1% FBS and finally incubated for 2 hours with MAP-2 (mouse, 1/1000 in PBS) and Cytochrome C (rabbit, 1/100 in PBS) antibodies. Neuron survival (MAP-2, # neurons), neurite network (MAP-2, total neurite length in μ m) and Cytochrome C release in MAP-2(+) neurons (overlap MAP-2/CytC, in μ m²) was analyzed using automatic computer-based image analysis (30 pictures/well, 20x magnification, Operetta®, Perkin Elmer).

Results: Untreated rat dissociated cortical neuron cultures (control, no glutamate injury) was compared to glutamate-injury (20 μ M) alone, or in combination with pre/post-treatment with ACOU082 (10/30/100/300/1000 nM), retigabine (3/10 μ M) and BDNF (50 ng/mL). Glutamate injury alone reduced neuronal survival (63.5 $\pm$ 1.2%) and total neurite length (65.3 $\pm$ 1.8%), while increasing cytochrome C release (157.0 $\pm$ 5.3%) compared to control. ACOU082 treatment concentration-dependently improved neuronal survival (statistically

significant for 30-100 nM, $70.5 \pm 3.1\%$ and $71.0 \pm 1.3\%$) and preserved neurites (statistically significant for 30-300 nM, $80.3 \pm 3.5\%$ to $83.9 \pm 1.3\%$) vs glutamate alone. ACOU082 treatment statistically significantly decreased cytochrome C release vs glutamate alone across all tested concentrations except 10 nM ($91.6 \pm 5.1\%$ to $109.8 \pm 11.5\%$). Retigabine only had a statistically significant effect on cytochrome C release ($94.2 \pm 6.1\%$ and $95.2 \pm 5.5\%$), while BDNF as expected significantly increased neuronal survival ($91.9 \pm 1.1\%$) and preserved neurites ($86.9 \pm 1.7\%$) while reducing cytochrome C release ($77.7 \pm 2.8\%$).

Conclusions: These results clearly demonstrate a neuroprotective potential of ACOU082 with reduced cytochrome C release from mitochondria after excitotoxic insult, in addition to the previously reported otoprotective benefits in different hearing loss models. In addition to a potential for CNS benefits with systemic treatment, this also suggests potential treatment benefits in hearing loss from Kv7.4 activators on SGNs and neurites in the cochlea.

SA116. Subtype-Specific Vulnerability of Spiral Ganglion Neurons Across Cochlear Injury Models

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Background: Type I spiral ganglion neurons (SGNs), which synapse with inner hair cells (IHCs), are essential for auditory perception. Recent transcriptomic and physiological studies have subdivided them into three molecularly and functionally distinct subtypes (Ia, Ib, and Ic), characterized by differences in spontaneous firing rates and threshold sensitivities. These subtypes are not fully established at birth but undergo postnatal maturation, a process dependent on afferent input from IHCs. Type I SGNs degenerate in response to cochlear insults such as ototoxic drugs and aging; however, whether specific subtypes exhibit differential vulnerability remains unclear. This study investigated subtype-specific susceptibility using aminoglycoside ototoxicity and genetic hair cell ablation models.

Methods: Cochlear injury was induced in adult mice by two approaches: (1) aminoglycoside treatment—gentamicin injection into the posterior semicircular canal or systemic co-administration of kanamycin and furosemide in C57BL/6J mice; and (2) genetic ablation—Pou4f3-DTR/+ mice received intramuscular diphtheria toxin to selectively ablate hair cells. Mice were sacrificed 4–8 weeks post-treatment, and cochleae were processed for cryosectioning and immunohistochemistry. SGN subtypes were identified using antibodies against POU4F1 (type Ic), GATA3 (type II marker, aiding subtype delineation), and TUJ1 (pan-type I). The proportion of POU4F1-positive SGNs among total type I SGNs was quantified across cochlear regions.

Results: Both gentamicin and kanamycin–furosemide treatment resulted in significant type I SGN loss. Importantly, type Ic SGNs, identified by decreased POU4F1 expression, showed a disproportionately greater reduction than other subtypes, indicating preferential vulnerability. In contrast, Pou4f3-DTR mice exhibited overall SGN degeneration, but the proportion of type Ic neurons remained stable, suggesting non-preferential loss. These findings support the hypothesis that aminoglycosides exert both indirect effects (secondary to hair cell loss) and direct cellular stress (e.g., mitochondrial dysfunction, excitotoxicity), rendering Ic neurons particularly vulnerable. In contrast, genetic ablation produces uniform loss of trophic support, leading to subtype-independent degeneration.

Conclusions: Subtype-specific vulnerability of SGNs is dependent on the nature of cochlear injury. Type Ic SGNs are selectively susceptible to aminoglycoside ototoxicity, whereas

genetic hair cell ablation causes uniform degeneration across subtypes. Recognition of SGN subtype diversity is essential for understanding cochlear pathology and for developing targeted neuroprotective strategies.

SA117. The Time Course of MHCII Expression and SGN Degeneration in Mice After Hair Cell Death

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Background: Spiral ganglion neurons (SGNs) receive auditory information from cochlear hair cells, the auditory receptor cells, and transmit it to the CNS. SGNs gradually degenerate after hair cell death, potentially adversely affecting the efficacy of cochlear implants. While an inflammatory-immune response in the cochlea appears to be causal to neurodegeneration, it remains unclear which components of the immune response are responsible. Gene expression profiling shows dramatic upregulation of immune response-related genes, including MHCII, a molecule involved in antigen presentation to lymphocytes and adaptive immune response, in the spiral ganglion following deafening. Preliminary data showed the MHCII knockout prevents SGN degeneration, indicating a causal role of MHCII-mediated antigen presentation and adaptive immune response in SGN loss. Here we ask whether the timing of MHCII expression post-deafening is correlated with the timecourse of SGN death in a mouse model of hair cell loss.

Methods: Male and female Pou4f3+/huDTR CBA/CaJ mice (n=24) were intramuscularly injected with a single dose of diphtheria toxin (DT) (5 ng/g) at postnatal day 5, which causes a rapid loss of hair cells. Mice were euthanized at postnatal days 13, 32, 39 and 60 for histological examination of the spiral ganglia to count surviving SGNs, and to detect MHCII expression. Cochlear sections were immunolabeled with the following antibodies: Tuj1 to label SGNs, anti-MHCII antibodies and anti-F4/80 to label macrophages. Quantitative analysis was done on every cochlear turn in every fourth near-mid-modiolar section. Image analysis was performed using IMARIS software. The outline of the Rosenthal canal for each turn was manually traced to measure the cross-sectional area and to calculate cell densities for SGNs and macrophages.

Results: At postnatal days 13, 32 and 39, the number of SGNs and MHCII+ macrophages in DT-deafened mice is not significantly different from control hearing mice. Correspondingly, the number of MHCII-expressing macrophages is not significantly different from that in control hearing mice. In DT-deafened mice at postnatal day 60, the number of SGNs has decreased and the number of MHCII-expressing (MHCII+) macrophages has increased, relative to hearing controls, indicating that between postnatal days 39 and 60, MHCII expression increases and SGN death becomes significant.

Conclusions: We demonstrated by immunolabeling that MHCII expression appears to correlate temporally with SGN degeneration. At P13, P32 and P39, post-deafening SGN degeneration has not yet occurred and upregulation of MHCII expression is not apparent. At P60, SGN degeneration is apparent after hair cell loss as is increased MHCII expression. These results are consistent with a causal association between MHCII expression on spiral ganglion macrophages and SGN degeneration. The function of MHCII expression in this context remains to be elucidated.

SA118. Targeting Nitritative Stress Protects Against Noise-Induced Cochlear Synaptopathy and Hearing Loss

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Background: Noise-induced hearing loss (NIHL) represents a significant public health issue, with limited therapeutic strategies available. Emerging evidence implicates nitritative stress, driven by excessive peroxynitrite generation, in the pathogenesis of cochlear injury. However, the role of nitritative stress as a potential target for preventing noise-induced hearing loss is not fully defined. This study evaluated the otoprotective potential of MnTBAP, a peroxynitrite scavenging metalloporphyrin, against noise-induced cochlear synaptopathy and hearing loss in mice.

Methods: Six-week-old CBA/J mice of either sex were exposed to 90 dB SPL broadband noise for 2 hours daily for two weeks. Permanent threshold shifts were assessed using auditory brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAE) two weeks after the final noise exposure. ABR wave-I amplitude and latency were analyzed as markers of cochlear synaptopathy. To inhibit nitritative stress, mice were treated with daily intraperitoneal injections of MnTBAP (10 mg/kg) for four weeks.

Results: Noise exposure induced a 13 to 18 dB increase in hearing thresholds across multiple frequencies, indicating hearing impairment. Specifically, ABR thresholds were elevated in response to pure-tone stimuli at 4 ($p = 0.002$), 8 ($p = 0.01$), 16 ($p = 0.001$), 24 ($p = 0.01$), and 32 kHz ($p = 0.0005$), as well as click ($p = 0.01$) stimuli. The shift in hearing thresholds was most pronounced at higher frequency (32 kHz) stimuli. OHC function, as measured by DPOAEs, was impaired at 16 ($p = 0.001$), 24 ($p = 0.002$), and 32 kHz ($p = 0.003$). Measurement of ABR wave-I amplitude and latency recorded with pure-tone and click stimuli at 90 dB SPL indicated that noise exposure induced a significant reduction in wave-I amplitudes at click ($p = 0.02$), 24 ($p = 0.0001$) and 32 kHz ($p = 0.0001$) frequencies, indicating impaired auditory nerve activity. Further, the ABR wave-I latency was increased at 24 ($p = 0.0001$), and 32 kHz ($p = 0.0006$) frequencies after noise exposure, showing delayed neural transmission. However, MnTBAP treatment significantly attenuated noise-induced increases in hearing thresholds, loss of OHC function, and cochlear synaptopathy indicating the otoprotective effects of MnTBAP.

Conclusions: Overall, the findings of this study indicate that noise exposure caused hearing loss, OHC dysfunction, and cochlear synaptopathy in mice, which is most severe at higher frequencies. MnTBAP protected against noise-induced auditory dysfunction, indicating that nitritative stress plays a central role in NIHL. These results suggest that peroxynitrite scavenging is a potential interventional strategy to prevent NIHL.

SA119. Screening of Piperlongumine Derivatives Against Excitotoxic Damage

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Background: According to the World Health Organization, over 500 million people worldwide live with disabling hearing loss. Despite its profound social and economic impact, the U.S. Food and Drug Administration (FDA) has only recently approved a therapy for

cisplatin-induced ototoxicity, and even then, only for a limited subset of cancer patients. Among the leading causes of acquired hearing loss is excitatory damage from noise exposure. Previous studies from our laboratory identified piperlongumine (PG), a natural compound derived from the Indian long pepper (*Piper longum*), as a promising therapeutic candidate for treating aminoglycoside- and noise-induced hearing loss. Building on this work, we synthesized a second generation of PG derivatives and tested them in a zebrafish model of excitotoxicity. We hypothesize modifications in the PG structure will result in more potent derivatives capable of offering greater protection against excitotoxic trauma.

Methods: We screened 30 PG derivatives using a zebrafish model of excitotoxicity. Zebrafish larvae (5–6 days post-fertilization) were exposed to 600 μ M kainic acid (KA), followed by treatment with one of the PG derivatives. After treatment, the larvae were fixed and immunostained with otoferlin, a hair cell marker, and hair cells were manually counted under a fluorescence microscope.

The top three lead compounds, PG-12, PG-23, and PG-24, were selected for further analysis, which included: 1) dose-response curve generation, 2) quantification of ribbon synapses in the presence of KA and the PG derivatives, and 3) FM1-43 uptake assay to assess mechanotransduction channel activity. Data were expressed as Mean $\pm$ SD, and statistical analyses were performed using Student's t-test or one-way ANOVA followed by Dunnett's post hoc test for multiple comparisons. Results were compared against both vehicle control and kainic acid-only groups.

Results: The majority of the PG derivatives showed protective effects against excitotoxicity at least one concentration. Notably, PG-12, PG-23 and PG-24, demonstrated consistent protection across multiple concentrations. Dose-response analysis revealed sub-nanomolar EC₅₀ values, indicating high potency. Evaluation of ribbon synapses and FM1-43 uptake is currently underway.

Conclusions: By modifying piperlongumine chemical structure we were able to obtain more effective compounds at protecting against KA-induced excitotoxicity. Ongoing and future experiments will explore the efficacy of these lead derivatives in more clinically relevant models of hearing loss.

SA120. Surface Mild Hypothermia for Acute Noise-Induced Hearing Protection in Firefighters

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Background: Noise-induced hearing loss (NIHL) remains a significant occupational hazard for firefighters due to repeated exposure to hazardous noise during active duty. Acute noise exposure triggers inflammatory responses within the cochlea, contributing to hair cell injury and auditory dysfunction. Timely intervention is challenging in real-world settings, limiting opportunities to medicate potential noise induced damage. Surface-applied mild therapeutic hypothermia (MTH) may offer a practical approach to deliver rapid otoprotection in real time and potentially preserving hearing. This study aims to assess the safety, feasibility, and potential protective effects of applied MTH using the ReBound™ device in noise-exposed firefighters, with an emphasis on acute auditory changes and selective frequency protection.

Methods: Twenty-six adult on-duty firefighters (ages 18–55) were recruited. Inclusion required normal tympanometry and no active otologic pathology. Baseline audiometry was performed immediately following 24-hour on-duty shifts, followed by 20 minutes of surface cooling with the ReBound™ device and immediate post-cooling repeat audiometric assessment. Outcomes were compared to a non-noise-exposed control group (n = 25). Participants were monitored for 24 hours post-intervention for safety and tolerability.

Results: Pre- to post-cooling audiometric scores improved significantly across frequencies ($F(1, N) = 49.25, p < .001, \eta^2 = 0.534$), with no significant difference between control and experimental groups ($F(1, N) = 1.33, p = .256, \eta^2 = 0.03$). Scores varied across frequency measures ($F(6, N) = 7.76, p < .001, \eta^2 = 0.744$), and pre-post change differed by frequency ($F(6, N) = 4.26, p < .001, \eta^2 = 0.662$), suggesting selective protective effects at specific frequencies. The Time $\times$ Frequencies $\times$ Group interaction was not significant ($p = .247, \eta^2 = 0.334$). No adverse events were observed during 24-hour follow-up, demonstrating safety and tolerability of the ReBound™ device.

Conclusions: Applied surface MTH via the ReBound™ device is safe, feasible, and shows potential protective effects against acute noise-induced auditory changes in firefighters. Improvements were frequency-dependent, suggesting targeted mitigation of cochlear injury. These findings support further investigation of surface hypothermia as a rapid, non-invasive therapeutic approach for occupational NIHL, with potential translational applications for early intervention following hazardous noise exposure.

SA121. Exploring the Relationship Between the Two Most Abundant Outer Hair Cell Proteins: Prestin and Oncomodulin

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Background: Outer hair cells (OHCs) exhibit somatic electromotility, which is essential for high sensitivity and precise frequency selectivity in hearing. Prestin is the key molecule responsible for the electromotility of OHCs. As the most abundant membrane protein in OHCs, prestin occupies a significant portion of the lateral membrane. Prestin-KO mice exhibit a 40-50 dB shift in hearing thresholds, with their OHCs ~40% smaller than those of WT mice. Oncomodulin (OCM) is the most abundant cytoplasmic protein in OHCs and serves as a Ca²⁺-buffer. The absence of OCM leads to progressive hearing loss and degeneration of OHCs. Both prestin and OCM start to be expressed in OHCs during a similar developmental period, and their expression is regulated by microRNA 96 and the transcription factor Ikzf2. Since prestin's function is affected by Ca²⁺ signaling, there has been a long-standing hypothesis that these two abundant proteins in OHCs might interact and influence each other's roles for normal hearing. To determine whether the absence of OCM affects prestin's function, we used our newly established *Ocm*-KO mouse model to conduct hearing tests and nonlinear capacitance (NLC) measurements in OHCs. NLC is an electrophysiological technique that allows us to evaluate the functionality of prestin.

Methods: We created a new *Ocm*-KO mouse model, *Ocmtm1a/m1a*, using the 'KO-first, conditional-ready' strategy. Hearing was assessed by measuring ABRs and DPOAEs from 1 month to 12 months. Whole-cell recordings were performed in isolated OHCs obtained from *Ocm*-KO and WT littermates (P15–109). NLC data were analyzed by a two-state Boltzmann equation. Isolated OHCs were also visually inspected for morphological abnormalities.

Results: Significantly more OHCs from Ocm-KO mice exhibit atypical morphology compared to those from WT mice after sample preparation for NLC measurement. At P15, OHCs from both WT and Ocm-KO mice have similar NLC values and cell sizes. ABR and DPOAE thresholds of Ocm-KO were WT-like up to 2 months. However, the NLC and OHC size of Ocm-KO were significantly smaller compared to WT at 1 month, although the voltage sensitivity and operating point remained WT-like range from P15 to P109.

Conclusions: The atypical morphology found in OHCs isolated from Ocm-KO mice indicates that these OHCs are more sensitive to chemical/mechanical perturbations, highlighting the importance of OCM for OHC maintenance. The OHCs without OCM begin to show detectable atrophic changes (smaller size) and functional declines (reduced NLC) despite Ocm-KO mice having normal hearing. Since reductions in the sizes of OHCs and NLC are signs of aging OHCs, it remains unclear whether the absence of OCM accelerates the aging of OHCs, resulting in a decrease in prestin, or if the reduction of prestin in OHCs of Ocm-KO is what leads to OHC aging. (Work supported by the Knowles Leadership Fund and NIH R56DC020542 to JZ)

SA122. Can Oncomodulin Act as a Stress Sensor?

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Background: Outer hair cells (OHCs) are the first to suffer from elevated intracellular Ca²⁺ and reactive oxygen species (ROS). To maintain Ca²⁺ homeostasis, OHCs rely on various proteins, the most abundant being oncomodulin (OCM). The absence of OCM in mice leads to progressive hearing loss and OHC degeneration. OCM has been recognized as a primary intracellular Ca²⁺ buffer in OHCs. However, OCM can undergo significant conformational changes between its Ca²⁺-bound and Ca²⁺-free states. Furthermore, OCM is suggested to act as a redox sensor, as oxidizing conditions promote its dimerization through intermolecular disulfide bonding via the Cys18 thiol, and Ca²⁺-bound OCM accelerates this dimer formation. Given that structural alterations are common features of both Ca²⁺ sensors and redox sensors, OCM's properties likely enable it to function as a stress sensor for elevated Ca²⁺ and ROS, which are two common causes of OHC loss. However, little has been studied about OCM's role as a stress sensor or how it responds to stress in OHCs. Our aim is to explore OCM's function as a stress sensor using both in vitro cell line and in vivo mouse studies.

Methods: (1) In vitro system: We transfected OK cells with a plasmid containing the Ocm gene to establish a stable cell line that expresses Myc-DDK-tagged Ocm. We then treated the Ocm-expressing cells with 10 μ M A23187 for 30 minutes. A23187 is a known stressor that increases cytosolic calcium levels. (2) In vivo system: We administered a single intravenous dose of 16 mg/kg cisplatin to WT mice. Cisplatin increases ROS levels in different cells including OHCs. Following treatment, we collected both the A23187- and cisplatin-treated samples, as well as their solvent control counterparts, for SDS-PAGE/Western blotting using an antibody specific to either OCM or Myc. Non-transfected OK cells and cochleae from Ocm-KO mice were used as a negative control for Western blot.

Results: (1) Ocm-expressing cells treated with A23187 showed an increased ratio of OCM dimer to the total of monomer and dimer compared to non-treated Ocm-expressing cells. The OCM band was undetected in the non-transfected OK cells. (2) Cisplatin-treated WT cochleae displayed a higher ratio of OCM dimer to the combined amount of monomer and dimer compared to untreated WT cochleae. The OCM dimer band disappeared when the

reducing agent DTT was added to the cochlear samples, indicating that disulfide bonding likely plays a role in OCM dimer formation. Additionally, the OCM band was not detectable in Ocm-KO cochleae.

Conclusions: These results indicate that stressful stimuli enhance intracellular OCM dimerization, which suggests that OCM may function as a stress sensor for both increased Ca²⁺ and ROS levels. (Work supported by the Knowles Leadership Fund and NIH R56DC020542 to JZ).

SA123. Hair Cell Regulation of the Stereociliary PMCA2 Ca²⁺ Pump Density

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Background: Sound transduction in cochlear hair cells arises by activation of mechano-electrical transducer (MET) channels for which transmembrane channel-like protein 1 (TMC1) is the pore forming subunit. We made mice with mutations in Tmc1 that culminate in hair cell apoptosis and deafness by postnatal day (P) 21. However, mechano-transduction was normal at P6, when TMC1 is fully expressed. The goal is to understand when and how apoptosis is triggered. We hypothesized that apoptotic behavior may stem from an increase in cytoplasmic Ca²⁺.

Methods: We studied four mutations, Tmc1 p.D569N, p.M412K, p.T416K and p.D528N. MET channels in all mutants were gated normally, but with diminished Ca²⁺ permeability at P6, T416K being the least abnormal and D528N the most. Early signs of hair cell apoptosis were assayed in neonatal Tmc1 mutants by labeling with MitoTracker and Annexin V, the latter marking scramblase externalization of phosphatidyl serine. Reduced labeling with MitoTracker indicated degraded mitochondrial function in Tmc1 mutants, even at P6 when MET currents were still present. The reduction in MitoTracker labeling was correlated with the reduced Ca²⁺ permeability of the MET channel. Acoustic brainstem responses established that Tmc1 p.D569N, p.D528N and p.M412K mice were deaf by P15 but hearing was retained in Tmc1 p.T416K until P21. Stereociliary PMCA2 was determined by immunolabeling with the NR2 antibody and visualizing by confocal microscopy.

Results: We tested whether apoptotic behavior was influenced by blocking the stereociliary PMCA2 CaATPase pump to abolish Ca²⁺ extrusion. Scramblase activity was stimulated and MitoTracker labeling diminished by raising external pH from 7.4 to 9.0 or by adding 50 micromolar carboxyeosin to block PMCA2, suggesting apoptosis is triggered by increased hair cell [Ca²⁺]. Reduced PMCA2 in stereocilia was found for all Tmc1 mutants and was correlated with a decrease in Ca²⁺ influx via MET channels ($r = 0.73$). However, in Tmc1;Tmc2 double knockouts with zero Ca²⁺ influx, there was still a residual PMCA2 expression. PMCA2 expression was also increased after birth in parallel with development of the MET current. To directly test Ca²⁺ involvement, isolated cochleae were cultured in reduced (0.45 mM) external Ca²⁺ for 1 hour. This significantly lowered PMCA2 density as did perfusion with 4 micromolar curare to half-block the MET channels. These manipulations influenced PMCA2 density up to P11 but not after the onset of hearing. PMCA2 turnover at P6 was insensitive to cycloheximide, a protein synthesis inhibitor, but was much diminished by Pitstop2, a blocker of endocytosis.

Conclusions: The path connecting Tmc1 mutation to reduced PMCA2 reflects a homeostatic mechanism during the neonatal period due to reduced Ca²⁺ permeability in the mutants. The

results suggest a connection between stereociliary PMCA2 density, hair cell apoptosis and deafness. Funded by grant R01DC01362 from NIDCD

SA124. Dimensions of a Mature Living Cochlear Inner-Hair-Cell Bundle

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Background: Cochlear hair cells possess actin-filled membrane protrusions at their apical surface called stereocilia. Stereocilia are arranged stepwise into rows from tallest to shortest, forming the mechanotransducing hair bundle. The mechanotransducing function of the hair bundle is regulated by its dimensions, which include stereocilium heights and widths, the number of stereocilia per row, the distances between the stereocilia, and the shapes of the rows. Previously, we generated a blueprint of a living cochlear inner-hair-cell bundle for a P11 mouse (Miller et al., 2021, Front Cell Dev Biol). By comparing measurements in living and fixed tissues, we generated conversion factors that enabled us to impute living bundle dimensions from lightly-fixed phalloidin-labeled tissue imaged using confocal microscopy and from strongly-fixed tissue imaged using scanning electron microscopy (SEM). Inner-hair-cell bundles continue to change after P11 and are thought to finish development before P31. In this study, we measured the dimensions of P31 inner-hair-cell bundles using multiple imaging techniques. We then compared the dimensions of the mature P31 bundle with those of the immature P11 bundle.

Methods: Comprehensive measurements were performed using three techniques: fluorescent imaging of live inner-hair-cell bundles stained with membrane dye (FM-143), fluorescent imaging of lightly-fixed hair bundles with actin labeled by phalloidin-Alexa 488, and SEM imaging of strongly-fixed bundles. We compared these measurements to determine conversion factors for the dimensions obtained using each technique. Using these factors, we determined the complete set of dimensions for P31 inner-hair-cell hair bundles, focusing on the 90th to 160th inner-hair-cell bundles from the cochlear apex.

Results: Live P31 inner-hair-cell bundle dimensions were either measured directly or imputed using SEM measurements in strongly-fixed tissues and the conversion factors between strongly-fixed and live tissues. This enabled us to generate the first complete blueprint of a mature living cochlear bundle. Comparing the P31 blueprint with the P11 blueprint from the same tonotopical position revealed critical developmental changes. We evaluated the biophysical consequences of these changes using mathematical models.

Conclusions: The dimensions of a hair bundle – stereocilium height, width, number, and spacing, as well as row shape – directly regulate hair-bundle function. These dimensions differ between hair-bundle types, species, cochlear locations, and developmental age. We determined the dimensions of a mature inner-hair-cell bundle and compared the dimensions with those of an immature bundle. By precisely determining bundle dimensions and how they change during development, we gain a better understanding of how hair-bundle structure regulates its function in mammals.

SA125. IQGAP1 Deficiency Induces Progressive Hearing Loss With Early Onset

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Background: IQ motif-containing GTPase-activating protein 1 (IQGAP1) is a scaffolding protein that is ubiquitously expressed. It has roles in many different aspects in cell physiology and interacts with numerous proteins. Recent studies have shown a direct interaction between IQGAP1 and proteins coded by genes known to be related to hearing loss (Pendrin, Pejvakian). To investigate the role of IQGAP1 in hearing function, we used an *Iqgap1* knock-out (*Iqgap1*^{-/-}) mouse model.

Methods: In littermate *Iqgap1*^{-/-}, *Iqgap1*^{+/-} and wild-type (WT) mice aged 1 to 12 months, we tested auditory function by measuring auditory brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAE) at regular time intervals. We also evaluated IQGAP1 expression using Western blot and immunolabeling methods. Cochlear hair cell loss was quantified by immunolabeling methods at 12 months of age.

Results: Our results show that *Iqgap1* is highly expressed in supporting cells of the organ of Corti and in the spiral ligament and stria vascularis cells. Young *Iqgap1*^{-/-} mice (1-month-old) display a significant sensorineural hearing loss as evidenced by increased ABR thresholds at the highest (24 kHz, 32 kHz) and lowest (6 kHz) frequencies, associated with a reduced amplitude of DPOAEs. In addition, *Iqgap1*^{-/-} mice show auditory pathway impairment characterized by reduced amplitude and increased latency as measured by ABR. By 6 months of age, the ABR threshold shift and DPOAE amplitude reduction have progressed to impact all frequencies. Morphological evaluation shows substantial apical OHC loss in 12-month-old *Iqgap1*^{-/-} mice compared to age-matched *Iqgap1*^{+/-} and WT controls.

Conclusions: These results demonstrate an essential role for IQGAP1 in cochlear physiology and support its requirement for the maintenance of hearing function and OHC survival during aging.

Acknowledgment: This research was supported in part by NIH-NIDCD R01s DC002842, DC012049, and DC017955 (RJHS).

SA126. CIB2 Regulates Mechano-Electrical Transducer Channels in Mammalian Auditory Hair Cells via Plasma Membrane-Associated Proteins

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Background: Calcium and Integrin-Binding protein 2 (CIB2) is essential for hearing and mechano-electrical transduction (MET) in auditory hair cells (Riazuddin et al., 2012; Giese et al., 2017). CIB2 interacts with TMC1/2 (Giese et al., 2017), pore-forming subunits of the MET channel and is thought to be essential for their transport or retention at stereocilia tips (Liang et al., 2021). Multiple interacting sites between CIB2 and TMCs (Giese et al., 2024; Wu et al., 2025; Li et al., 2025) suggest that CIB2 is an accessory subunit to the MET channel. Thus, in addition to TMCs localization, CIB2 may regulate MET channel activity.

Methods: We generated a new mouse knock-in strain carrying the human deafness-associated variant p.R186W that does not completely abolish CIB2-TMC1/2 interactions, hence, retains some MET currents. Auditory brainstem responses were recorded to determine hearing function. Whole-cell patch clamp recordings assessed MET currents in P4-P7 auditory hair cells. Besides conventional deflection of hair cell bundles with fluid-jet, we used a custom piezo-driven probe capable of deflecting stereocilia within $\sim 5\mu\text{s}$ (confirmed by video recordings at 90,000 fps), which was faster than the speed of MET current activation. Focused-Ion Beam Scanning Electron Microscopy (FIB-SEM) was used to determine ultrastructural changes at the tips of transducing stereocilia produced by the p.R186W variant. Immunofluorescence labeling and confocal imaging were used to investigate localization of known proteins regulating MET function. To confirm the molecular mechanisms, we recapitulated p.R186W effects in wildtype hair cells by mis-localizing some stereocilia proteins with the MET channel blocker tubocurarine and compared p.R186W effects with the ones we recorded in *Tmc1*^{-/-} mice.

Results: Consistent with prior reports, the *Cib2*R186W/R186W mice were profoundly deaf at P16. However, our data are not consistent with a simple decrease of TMC1 in stereocilia but show more profound effects of p.R186W on the MET currents, stereocilia membrane composition, and its ultrastructure. Both inner and outer hair cells of *Cib2*R186W/R186W mice showed larger resting current and steeper MET current-displacement curves, suggesting increased force sensitivity. The p.R186W variant also slowed down MET current activation and eliminated fast adaptation, even though the MET current dependence on intracellular potential (presumably driven by Ca^{2+}) remained intact. Both effects were consistent with the mis-localization of PIP2 observed in *Cib2*R186W/R186W stereocilia. Additionally, we found disrupted lower tip-link densities in second-row stereocilia and mis-localization of the membrane-associated protein BAIAP2L2 from *Cib2*R186W/R186W but not in *Tmc1*^{-/-} stereocilia tips. Pharmacological mis-localization of BAIAP2L2 with tubocurarine in wildtype mice recapitulated the p.R186W effects on MET current activation and force sensitivity.

Conclusions: We concluded that p.R186W variant disrupts the properties of plasma membrane by mis-localizing BAIAP2L2 and PIP2 thus affecting force transmission to the MET channels.

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SA127. The Temporal Coincidence Between Nuclear Translocation and BK Channel Expression in Mouse Inner Hair Cells

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Background: Nuclear position is tightly regulated during cell division, migration, and maturation. We previously identified a programmed nuclear translocation in the cochlear inner hair cells (IHCs) of rat and mouse that coincides with their functional maturation around hearing onset. In early postnatal days, the nucleus resides at the basal pole of the IHC—distant from the apical stereocilia but close to nascent afferent and efferent synapses—during a phase of rapid cell growth. After the IHC reaches its final length, the nucleus shifts to a mid-cellular position. Nuclear migration coincides with the surface expression and aggregation of BK channel plaques and signals the final phase of functional maturation. Here, our goal was to define the timeline and relationship between nuclear migration and

expression of BK channels, toward understanding the mechanisms regulating this critical phase of hair cell maturation.

Methods: We examined BK channel protein expression, surface localization, and their relationship to nuclear position in inner hair cells using immunolabeling and western blot analysis. Cochleae were collected from C57BL/6 wildtype mice at P10–P14 and P26, with denser sampling near P12. BK channels were labeled with rabbit anti-BK, cytoplasm with mouse anti-myosin VII or anti-vglut3, and nuclei with DAPI. High-resolution confocal imaging was used to reconstruct inner hair cell morphology and quantify cell size, nuclear position, and BK channel plaque distribution. Western blots for BK channels were performed at intervals from P8 to P14. To perturb nuclear positioning, a subset of animals received AAV-SYNE4 injections at P0–P2. SYNE4 is a gene encoding a nuclear complex linker protein known to be found in the nuclear envelopes of inner and outer hair cells. After auditory function was assessed using ABR and DPOAE, cochleae were fixed and immunolabeled as described above. Antibody specificity and viral transduction were consistent with expected structures.

Results: Western blot and immunohistochemical analysis revealed that BK channel proteins are present in inner hair cells well before hearing onset and before their functional insertion into the membrane. Syne 4 overexpression did not ubiquitously prevent the surface expression of BK channels. However, overexpression of SYNE 4 disrupted the normal nuclear positioning of inner hair cells and altered the cytoskeletal distribution of Myosin VII. These morphological changes, along with a modest elevation in ABR thresholds, were observed in most SYNE4-injected animals but not in sham controls, suggesting that perturbing nuclear position in inner hair cells impacts auditory function.

Conclusions: Nuclear migration at the onset of hearing is a key step in the morphological maturation of IHCs. Our findings indicate that while nuclear positioning influences auditory function, BK channel surface expression is not explicitly dependent on nuclear migration. Their temporal coincidence during natural development suggests that both processes may rely on shared maturational milestones rather than being causally linked.

SA128. Modulation of IHC Calcium Channel Inactivation Kinetics by CaBP2 Variants

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Background: Voltage-gated calcium (CaV) channels undergo voltage- and calcium-dependent inactivation (VDI, CDI) to prevent excessive entry of calcium ions in the cells. In the cochlear inner hair cells (IHCs), and retinal photoreceptors, this inactivation is strongly attenuated to enable persistent signalling of sensory information. The factors that reduce the amount of channel inactivation include calcium-binding proteins (CaBPs), small molecules similar in structure to calmodulin. In the IHCs, CaBP2 and to a lesser extent CaBP1 are strongly expressed and mutations in CABP2 have been identified to cause nonsyndromic hearing impairment DFNB93 in the affected patients. We have previously investigated the phenotype of Cabp1/2 double KO animals and tested the feasibility of gene therapy in DFNB93 mouse models. Hearing of treated animals showed significant improvement upon

gene therapy, which we attributed to partial rescue of IHC CaV1.3 properties and of synaptic IHC exocytosis. Now, we would like to understand, how exactly CaBP2 exerts its function on the CaV1.3 calcium channel that mediates synaptic transmission in the IHCs.

Methods: Using a combination of in vitro biophysical, biochemical and electrophysiological methods and in vivo hearing measurements, we are investigating the effectiveness of different CaBP2 isoforms and mutants in attenuating channel inactivation, and the role of calcium binding for this function.

Results: We determined calcium binding of individual CaBP2 EF hand domains and identified CaBP2 variants with reduced effects on CaV1.3 channel properties.

Conclusions: Our data so far suggests that metal-ion binding of (at least some) EF hands and the N-segment are required to support proper CaV1.3 function, interaction with CaM and hearing.

SA129. Ectopic Mouse TMC1 and TMC2 Alone Form Mechanosensitive Channels That Are Potently Modulated by TMIE

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Background: The mechanotransduction (MT) channel in inner ear hair cells converts sound/head movements into electrochemical signals. TMC1/2 are pore-forming subunits, but functional studies in heterologous cells are limited by poor cell-surface expression. Prior work circumvented this by reconstituting truncated TMC1/2 in liposomes, but whether full-length mammalian TMC1/2 can form MS channels alone, or if TMIE is required, remained unknown. We aims to overcome expression issues and elucidate the function of full-length mammalian TMC1/2 and TMIE in mechanosensitive channel activity.

Methods: We engineered Fyn-TMC1 and Fyn-TMC2 by fusing at the N terminus of mTMC1/2 an 11-residue membrane-anchoring motif (MGCVQCKDKEA) derived from the Src kinase Fyn, which we named Fyn tag (Fig. 1A). This motif serves as a signal for myristoylation and palmitoylation, which aid stable anchoring of proteins to the plasma membrane and thereby promote their cell-surface expression. Then we cultured cell lines, including PIEZO1-knockout HEK293T cells, and transfected them with plasmids encoding mTMC1/2, hTMC1/2, mTMIE, and their mutants. Protein localization was assessed using confocal microscopy and immunostaining. Cell-surface biotinylation assays were performed to quantify protein expression on the cell membrane. Protein-protein interactions were assessed via pull-down assays. Channel activity was measured using whole-cell patch-clamp and single-channel recordings, with mechanical stimulation achieved by cell poking. Intracellular calcium levels were assessed using calcium imaging. Statistical analyses were used to validate the findings.

Results: 1. The Fyn lipidation tag enhances cell-surface expression of mouse and human TMC1/2 in heterologous cells.

2. Ectopically expressed full-length mTMC1/2 alone form functional mechanosensitive channels.

Mouse transmembrane inner ear (TMIE) protein robustly stimulates TMC1/2 channel activity by modulating their gating.

3. The N-terminal 27 residues of mTMIE are dispensable for regulating TMC1/2 in the in vitro functional assay.

4. Mutating mTMIE C76C77, the predicted palmitoylation sites, eliminates mTMIE stimulation of mTMC1/2.
5. mTMC1/2+mTMIE form 18 pS and 24 pS single channels, respectively.
6. mTMC1/2+mTMIE single channels showed biophysical and pharmacological properties similar to those of the MT channel.

Conclusions: This study demonstrates that the addition of a Fyn lipidation tag overcomes the technical hurdle of expressing TMC1/2 in heterologous cells, allowing for functional characterization. The findings confirm that TMC1/2 alone can form mechanosensitive channels and that TMIE enhances their activity through gating modulation. Palmitoylation of TMIE plays a critical role in this regulatory function. The observed single-channel properties align with those of native mechanotransduction channels. These results provide insights into the function of TMC1/2 and TMIE and establish a valuable in vitro assay for future investigations of the mechanotransduction complex.

SA130. Interrogating Domain Contributions to the Function of Transmembrane Channel-Like Proteins

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Background: Transmembrane channel protein-like 1 and 2 (TMC1/2) are the pore-forming subunits of the hair cell mechanosensory transduction (MT) complex, which transduces mechanical forces at the tips of stereocilia and initiates vertebrate hearing and balance. Despite both contributing to hair cell MT, TMC1/2 differ significantly in their ability to support auditory function, irrespective of their expression during development (Asai et al., 2018; Nakanishi et al., 2018). This suggests that intrinsic properties of TMC1/2 proteins drive their differing ability to sustain normal function in auditory hair cells. Interrogation of specific molecular domains of TMC1/2 may elucidate these differences, how they contribute to MT mechanistically, and what attributes are necessary to support auditory function.

Methods: Capitalizing on the strong homology shared between TMC1/2, we constructed protein chimeras targeting three domains identified with the highest sequence/structural divergence: the N-terminal region extending up to transmembrane helix TM1, the C-terminal region including TM10 that constitutes the TMC dimer interface, and the C-terminus extending to extracellular helices H7/H8, predicted to aid in the domain-swapped configuration of the TMC dimer. Vectors encoding these chimeras were packaged into hair cell-targeting AAVs (AAV2/AAV9-PHP.B), which were directly administered to P3-5 Tmc1/2KO cochlear explants or injected via the utricle into P1 Tmc1KO mice. Mechanosensory transduction of postnatal hair cells was assayed 48 hours after vector exposure via measurement of FM1-43 uptake. Audition was assessed in P25-30 mice by measuring ABR/DPOAEs. C-terminally tagged versions of each chimera were also assessed to evaluate the expression/localization of exogenous protein to the site of mechanosensory transduction.

Results: All TMC2 constructs with a chimeric TMC1 terminus successfully internalized FM1-43 and restored mechanosensory transduction (n=10 explants for XTmc1Nt-Tmc2/XTmc1TM10+Ct-Tmc2, n=2 for Tmc1Di-Tmc2). However, while early postnatal injections of these chimeras appear to promote increased hair cell survival compared to Tmc1KO controls, neither TMC2 with the TMC1 N-terminus (XTmc1Nt-Tmc2, n=6) nor the C-terminus up to TM10 (XTmc1TM10+Ct-Tmc2, n=2) rescued auditory function in adult

mice—both treatment groups displayed highly elevated to no ABR/DP thresholds. Almost none of the reciprocal TMC1 chimeras (XTmc2Nt-Tmc1/XTmc2TM10+Ct-Tmc1, n=10 each), despite tagged constructs properly localizing to stereocilia tips, were able to support hair cell MT and drive FM1-43 uptake. An exception was TMC1 with the TMC2 C-terminus extending to H7/H8 (XTmc2Di-Tmc1, n=2), which was found to restore hair cell MT and permit FM1-43 uptake in contrast to the chimera excluding H7/H8. This may point to the importance of the TMC dimer interface in recapitulating channel function in the TMC1 chimeras.

Conclusions: Our work identifies domain-specific requirements of TMCs in supporting both mechanosensory transduction and auditory function in the inner ear. Further work characterizing the channel features endowed by these domains will enhance our understanding of both TMC proteins, and channel properties important for the MT complex and greater auditory function.

SA131. Is the Distribution of Actin Isoforms Non-Uniform Within the Stereocilium Actin Core?

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Background: Auditory sensory hair cells have stereocilia at their apical surface, which are actin-based organelles that detect sound-induced vibrations. The stereocilium cytoskeleton has both beta- and gamma-actin isoforms. While hair cells can develop stereocilia bundles in the absence of either isoform, both actin isoforms are essential for the life-long maintenance of stereocilia. However, the ultrastructural organization and distribution of these actin isoforms within the tightly packed core of stereocilia is currently unknown. Here, we are testing the hypothesis that the stereocilium actin core exhibits small domains that are enriched in one isoform relative to the other.

Methods: Organ of Corti explants were isolated at early postnatal days from C57Bl/6 mice and from mice expressing fluorescent beta-actin in hair cells (FLE β -actin-eGFP x Atoh1CRE). Samples were imaged live or fixed and stained against filamentous actin with fluorescently-labeled phalloidin, or against specific actin isoforms with fluorescently-labeled specific antibodies. Samples were imaged using a Leica SP8 confocal microscope using the following objectives: Leica 100X 1.44 NA and Olympus 60X 1.0 NA or 100x 1.7 NA.

Results: Beta- and gamma-actin are both expressed along the entire stereocilium length in neonate auditory hair cells. However, using our highest resolution objective (1.7 NA), we see non-uniform labeling of beta- and gamma-actin along the stereocilia shafts, with small discrete zones appearing to be enriched in either beta- or gamma-actin. Standard deviations of fluorescence intensity profiles along the stereocilium shaft of stereocilia labeled with isoform-specific antibodies or of stereocilia from mice expressing beta-actin-eGFP were all comparable and significantly higher than those from stereocilia labeled for filamentous actin with phalloidin. We are currently evaluating the staining profiles of stereocilia simultaneously labeled with the same primary antibody but tagged with different fluorophores, or of stereocilia labeled with pan-actin antibodies.

Conclusions: Our preliminary results indicate that the stereocilium actin core is potentially made up of small domains enriched in beta- or gamma-actin. The functional significance of such non-uniform expression pattern of beta- and gamma-actin isoforms within stereocilia remains to be explored.

SA132. The Salamander Inner Ear: A Developmental and Comparative Study

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Background: Hearing loss in humans is usually permanent because damaged inner ear hair cells cannot regenerate. Salamanders, however, can replace lost hair cells, making them valuable models for sensory development and regeneration. Within the genus *Eurycea*, surface-dwelling (*E. nana*) and cave-dwelling (*E. rathbuni*) species exhibit contrasting developmental strategies and ecological adaptations, providing a natural comparative framework for studying inner ear development. The transcription factor PAX6, best known for its role in eye development, has recently been reported in hair cells of neuromast organs in *Eurycea* from a previous study in our lab. Building on these observations, we hypothesize that PAX6 is also present in inner ear hair cells, and that comparing its expression between surface and cave species may reveal conserved versus divergent roles in sensory development. To confirm hair cell identity, we will use MYO7A, a widely known hair cell marker. Double labeling with PAX6 and MYO7A will help us determine PAX6's presence in inner ear sensory cells across species.

Methods: We combined anatomical imaging with immunohistochemistry to compare the salamander inner ear of two species through development. Specimens at various developmental stages were iodine-stained and scanned with micro-CT (DiceCT) to reconstruct inner ear anatomy in 3D. To examine protein expression, specimens were cryosectioned and labeled with antibodies against PAX6 and MYO7A, followed by fluorescent secondary antibodies. Sections were imaged using laser-scanning confocal microscopy, enabling localization of proteins within inner ear hair cells across developmental stages.

Results: DiceCT images provided 3D reconstructions of inner ear anatomy in both *E. nana* and *E. rathbuni*, allowing comparison of overall structure across developmental stages. Immunofluorescence showed that PAX6 protein was localized in the sensory epithelium of the inner ear by the earliest stage examined (1 month post-oviposition) in both *E. nana* and *E. rathbuni*. Preliminary data indicate that PAX6 expression in the inner ear persists into later developmental stages, particularly in the cave-dwelling *E. rathbuni*. In confocal images, PAX6 labeling was localized to the putative hair-cell region of the inner ear, consistent with its presence in sensory cells. The MYO7A labeling is in progress to confirm which cells are hair cells and to assess their co-expression of PAX6.

Conclusions: These results establish that PAX6 is a component of salamander inner ear sensory cells from very early in development and may be maintained throughout life. By combining 3D imaging with molecular markers, we have started building a map of how the salamander's inner ear develops. Completing the MYO7A co-labeling will enable us to determine whether PAX6 is present in hair cells or support cells. This comparative developmental study lays the groundwork for understanding hair cell regeneration mechanisms and could inform strategies for restoring hearing function. Insights from this study may contribute to regenerative approaches for hearing loss.

SA133. Molecular Mechanisms of TMC1/2 Localization in Hair Cells Mediated by Transmembrane O-Methyltransferase (TOMT)

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Background: Transmembrane O-methyltransferase (TOMT) is a deafness-linked gene (DFNB63) necessary for hearing and mechanotransduction channel (MET) function in hair cells. Our previous work demonstrated that TOMT is critical for localization of the MET channel pore-forming subunits, TMC1 and TMC2, to the hair cell stereocilia, though endogenous expression patterns and molecular mechanisms of TOMT function are not well understood.

Methods: We generated a TOMT-HA knock-in mouse to evaluate inner ear TOMT expression and subcellular localization with immunohistochemistry. To gain mechanistic insights into the role of TOMT in regulating mechanotransduction and TMC1/2 localization, we created serial 20 amino acid deletions and deafness-causing missense mutation TOMT constructs to identify domains important for protein-protein interactions, normal and pathophysiological mechanisms, and auditory function. We performed co-immunoprecipitation of TOMT constructs with TMC1 and other MET associated proteins in HEK293 cells. To further evaluate TOMT function in vivo, we used adeno-associated viral (AAV) inner ear delivery of TOMT with deletion or deafness mutations into *Tomt*^{-/-} mice followed by immunohistochemistry and auditory brainstem response (ABR) testing to evaluate rescue of TMC1 localization and auditory function, respectively.

Results: We confirmed that endogenous TOMT expression is restricted to hair cells in the cochlea and vestibule and TOMT is localized to the hair cell endoplasmic reticulum (ER) and cytoplasm while excluded from stereocilia. We determined that deletion of the first 20 amino acids (2-21), a putative membrane-associated domain, abolishes binding of TOMT to TMC1 and other MET components. In contrast, deletions in other regions of TOMT and deafness-associated mutations in TOMT increase interaction with MET proteins. We found that the TOMT 2-21 region is critical for stereocilia localization of TMC1 and auditory function. In addition, though TOMT deafness mutations induced increased binding to MET channel components in vitro, they failed to rescue TMC1 localization to the stereocilia or ABRs. Interestingly, using in vitro cycloheximide chase assays, we found that the co-expression of TMC1 with mutant TOMT altered TMC1 protein stability.

Conclusions: In summary, TOMT is an ER-associated protein that is critical for TMC1/2 localization and auditory function. The 2-21 region of TOMT is necessary for interactions with MET channel proteins and TMC1 localization to stereocilia. In contrast, TOMT deafness-linked mutations increase binding to MET components, while failing to rescue TMC1 localization to the stereocilia, suggesting defects in MET protein complex dissociation.

SA134. Does Brief Membrane Permeabilization Cause Morphological Abnormalities in Stereocilia Hair Cell Bundles?

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Background: Detergents are amphipathic molecules often used to permeate the cell membrane to allow for better antibody penetration in immunolabeling experiments. This technique is particularly useful when it comes to antibodies that label the cytoskeleton and other small intracellular proteins. Permeabilization has also been used in live cells for functional labeling. Inner ear hair cells are specialized sensory cells that receive stimulus through unique microvilli-like protrusions called stereocilia. Individual stereocilia are generally less than 10 μm in height and 0.5 μm in diameter and contain an actin-based cytoskeleton. It is known that cytoskeleton remodeling happens in stereocilia as a result of changes in the influx of intracellular Ca^{2+} through mechano-electrical transduction channels (Velez-Ortega et al., Elife 2017). Thus, even a brief incubation in detergent and exposure to a different ionic environment, may be enough to trigger remodeling. Here we used several detergents under a variety of conditions to test if permeabilization affects the morphology of stereocilia hair bundles. Secondly we wanted to explore if a detergent treatment would expose the cytoskeleton of stereocilia for visualization using scanning electron microscopy (SEM).

Methods: Organ of Corti from C57Bl/6 early postnatal mice were dissected in L-15 medium and were treated with varying concentrations of detergent (Triton-X-100, Saponin, Tween-20, CHAPS, NP-40, SDS) before or after fixation. In live cells, treatments took place in different ionic environments (extracellular-like or intracellular-like). Morphology of stereocilia hair bundles was then assessed using SEM.

Results: As was expected there was significant damage to the hair cell bundle morphology, including thinning and potential remodeling of the cytoskeleton, with brief exposure to detergent before fixation in an extracellular-like (L-15 medium) environment. Live cells treated in an intracellular-like (cytoskeleton buffer) environment for up to 7 minutes had more normal hair cell bundle morphology, but there were exaggerated changes at the tips of stereocilia and hollow cores observed in supernumerary stereocilia. In all cases of brief exposure to detergent before fixation, there was an appearance of parallel filaments of approximately 15-20nm center-to-center. Furthermore, the kinocilium membrane was completely removed exposing the microtubule core. Membrane removal after fixation has proven difficult with no observed kinocilium membrane removal or appearance of parallel filaments along the stereocilia shafts.

Conclusions: Membrane removal is easier in live cells than cells that have been fixed prior to treatment with detergent. We are currently testing varied time points and conditions for treatment of samples with detergent prior to fixation. We hope to determine an effective treatment for permeation without causing significant morphological or remodeling changes in the stereocilia hair bundle.

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SA135. Simulations of Kanamycin A Permeation Through TMC1 Validate Its Role as the Inner-Ear Mechanotransduction Channel

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Background: Aminoglycosides are small-molecule antibiotics widely used as treatment against bacterial infections. A side-effect of aminoglycosides use is dose-dependent hearing loss. This is caused by death of specialized cells called hair-cells that are responsible for sensing sound. Multiple experiments involving manipulations of components of the

mechanotransduction machinery have shown that an important pathway for aminoglycoside intake by hair-cells is their transduction channel pore formed by transmembrane channel-like 1 (TMC1) proteins. Although experiments suggest that aminoglycosides enter hair cells through TMCs, the mechanism and the energetics of this process are poorly understood. We used a variety of computational methodologies to probe the mechanism of aminoglycoside permeation through TMC1.

Methods: We generated AlphaFold2 models of human TMC1 that had ten transmembrane helices labeled $\alpha 1$ to $\alpha 10$, with the pore formed by helices $\alpha 3$ to $\alpha 7$. These models were simulated with and without calcium-and integrin-binding protein 2 (CIB2) in the presence of Kanamycin A (KanA), in 3+ and 4+ charged states. Control simulations were done using curare, a known hair-cell transduction channel blocker. Multiple microsecond scale simulations of these systems were carried out in the presence of applied voltage to enable permeation. We also quantified the free energy of permeation by using an enhanced sampling technique that combines well-tempered metadynamics and extended adaptive biasing forces methods.

Results: Microsecond timescale simulations with applied voltage (-500 mV and -250 mV) showed complete permeation of KanA (3+ and 4+) and partial insertion of curare through the TMC1 pores. We also learnt that the movement of transmembrane helices $\alpha 3$ and $\alpha 4$ of TMC1 along with the formation of a kink in $\alpha 4$ were essential for permeation. These simulations also revealed crucial bottlenecks and interacting residues along the permeation path, some of which are at previously known deafness-causing mutation sites. Quantification of free energy of permeation of KanA 3+ across an open-like conformation of TMC1 system without CIB2 and in the absence of biasing voltages gave a barrier of ~ 10 kcal/mol. This barrier could be overcome in the presence of a resting voltage in the hair-cell environment.

Conclusions: The occurrence of permeation of KanA in simulations supports the notion that TMC1 proteins form the pore of the inner-ear hair-cell mechanotransduction channel. Simulations also demonstrate a potential channel opening mechanism involving the movement of transmembrane helices $\alpha 3$ and $\alpha 4$ of TMC1. Transient interactions formed by KanA with residues at previously known deafness-causing mutation sites provide insights into the roles of these residues in channel function. Average permeation times obtained from simulation repeats point to a more rigid pore of TMC1 in the presence of CIB2. Our work provides an improved understanding about aminoglycoside permeation through TMC1 that can inform the design of new molecules retaining their bactericidal activity while losing their ototoxicity.

SA136. Does Myosin-15a Isoform 1 Contribute to the “Gating Spring” of the Mechanotransduction Machinery in Mammalian Auditory Hair Cells?

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Background: Inner ear hair cells convert sound vibrations into electrical signals through deflections of their mechanosensory stereocilia. These deflections modulate tension in tip links interconnecting stereocilia and transmitting force to mechano-electrical transduction (MET) channels. The tip link and associated molecules form a “gating spring” that governs MET sensitivity (a stiffer spring allows channel opening with smaller deflection). Molecular identity of gating spring remains unknown. Notably, saturation of the MET current typically requires ~ 200 nm of stereocilia deflection, which likely exceeds the elastic limits of tip links. An additional elastic component, possibly a motor protein capable of large movements, may

contribute to the gating spring. Myosin-15a (MYO15A) is a motor protein required for stereocilia growth and maintenance, and mutations in MYO15A cause deafness. MYO15A can link the plasma membrane proteins/complexes to the stereocilia actin core, thereby contributing to the gating spring and providing the compliance needed for large deflections. Hair cells express three MYO15A isoforms. Notably, isoform 1 with an extended N-terminus is the largest variant that is localized at the tips of transducing, 2nd and 3rd row stereocilia, at least in the inner hair cells.

Methods: Using conventional whole-cell patch clamp recordings, we investigated MET currents in the young postnatal (P5-8) cochlear inner hair cells of wild-type and *Myo15 Δ 1/ Δ 1* (lacking MYO15A isoform 1) littermate mice. We used a custom fluid-jet (4.6 kHz piezo-driven, 5 μ m tip) for uniform hair bundle deflections. Hair bundle motion was captured at 2000 frames/s using a high-speed camera (Basler acA720-520 μ m) with a 60 $\times$ /1.0 NA water-immersion objective. We also re-analyzed MET recordings obtained previously in our lab in the same mice but using piezo-driven rigid probe deflecting stereocilia (Fang et al., 2025)

Results: Re-analysis of the previous data from our lab suggests that the lack of MYO15A isoform 1 causes MET currents to saturate at smaller deflections than in wild-type hair cells, indicating reduced gating spring compliance in the mutants. However, rigid probe stimulation is known to artificially broaden the deflection range and underestimate hair cell's mechanosensitivity. Using our fluid-jet system combined with simultaneous high-speed video recordings, we confirmed that MET currents saturate at $\sim$ 200 nm bundle deflection in wild-type inner hair cells, consistent with the previous reports. We were also able to reduce cell-to-cell variability, which could allow estimating the differences in the stiffness of the hair bundles in mutant and wildtype hair cells.

Conclusions: Our results indicate that the fluid-jet approach can be reliably used to test whether MYO15A isoform 1 contributes directly to the compliance of the gating spring.

SA137. Effects of cAMP on the Shape of Mammalian Auditory Hair Cells' Stereocilia

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Background: The exact heights and “staircase” arrangement of the three rows of actin-based mechanosensory stereocilia at the apical surface of the auditory hair cells are essential for their mechanosensitivity and, hence, our hearing. Sound-induced deflections of the hair bundles modulate the tension in extracellular tip links interconnecting stereocilia and gating mechano-electrical transduction (MET) channels located at the lower ends of the tip links. It has been established that the height of stereocilia may be dynamically regulated by the resting current passing through the MET channels; however, the exact molecular mechanisms of this phenomenon have yet to be elucidated. The second messenger cyclic adenosine 3'5'-monophosphate (cAMP) is known to modulate MET in the auditory hair cells and plays a role in the growth and/or disassembly of actin-based protrusions in other cell types. Therefore, this study aims to explore whether cAMP could modulate stereocilia heights in the auditory hair cells.

Methods: Two different experiments were performed: 1) organ of Corti explants dissected from postnatal day 4 (P4) wildtype mice were cultured for 2.5 days and then incubated for 6 hours at 37°C either with 200 μ M of membrane-permeable, nonhydrolyzable cAMP analog 8-Bromo-cAMP or only with the same intracellular medium (DMEM); 2) acutely isolated

organ of Corti explants from postnatal day 5 (P5) wildtype mice were incubated at 37°C with 10 mM of 8-Bromo-cAMP for 4 or 8 hours while the control samples were incubated in DMEM without 8-Bromo-cAMP for the same periods of time. Hair cell bundles were imaged by high-resolution scanning electron microscopy, and stereocilia dimensions were quantified using ImageJ.

Results: The preliminary data reveals that there were no significant changes in the step sizes between the three stereocilia rows after 4 hours exposure of acutely-isolated organs of Corti with a high concentration of 8-Bromo-cAMP. However, after 8 hours incubation at the same concentration, there was a decrease in the step size between the tallest and shortest row of stereocilia in both inner and outer hair cells. A similar effect was observed after 6 hours of incubation with lower concentration of 8-Bromo-cAMP but in cultured organs of Corti (P4 + 2.5 days in vitro). Furthermore, in the latter samples, there was a decrease in step size between the tallest and middle row in both inner and outer hair cells, while the heights of the tallest stereocilia in the bundle were not affected in outer hair cells but decreased in the inner hair cells.

Conclusions: These results imply that the effects of cAMP on stereocilia heights may either require cultured conditions or a certain developmental maturity of hair cells.

SA138. Single-Cell Proteomics and Ap-Ms to Elucidate Auditory Mechanotransduction in Mammalian Cochlea

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Background: The mammalian cochlea contains two mechanosensitive receptor cell types, inner hair cells (IHCs) and outer hair cells (OHCs), essential for hearing. Despite their importance, the molecular mechanisms driving their distinct function, particularly those underpinning mechano-electrical transduction (MET), remain incompletely understood. This study is the first effort to generate distinct proteomic profiles for IHCs and OHCs, using postnatal day 7–9 (P7–P9) murine cochleae, to uncover key proteins involved in auditory signalling, including novel components of the MET complex.

Methods: Due to the limited number and fragility of cochlear hair cells (~3,500 IHCs and ~12,000 OHCs per cochlea), we employed a suction pipette technique to isolate individual OHCs from enzymatically treated, mechanically dissociated tissue. Isolated cells were cryopreserved and processed using a streamlined one-pot protocol that combines lysis, sonication, denaturation, and tryptic digestion in a minimal volume. We applied TimsTOF SCP with Evosep One for mass spectrometry, identifying up to 500 protein groups per single OHC and 2,200 from pooled five-cell samples. Further analysis will use the higher-sensitivity TimsTOF Ultra 2 system.

Results: Initial work focused on OHCs due to their accessibility. Hair cell-specific proteins were successfully identified in OHCs. We now aim to compare them with recently isolated IHCs. To specifically study the MET complex, we integrate single-cell proteomic data with affinity-purification mass spectrometry (AP-MS) targeting the known MET component protocadherin 15 (Pcdh15). As Pcdh15 is an intrinsically disordered protein (IDP), we apply crosslinking strategies and optimized buffers to capture weak or transient interactions. Potentially interesting protein-protein interactions will be validated using AlphaFold3 and AlphaPulldown.

Conclusions: This study provides new molecular insights into cochlear mechanosensation by profiling key proteins and identifying components of the MET complex. Our approach advances single-cell proteomics in fragile cell types and contributes foundational knowledge for therapeutic strategies in auditory disorders.

SA139. Identifying Key Molecules Involved in the Biogenesis, Transport, and Recycling of Synaptic Vesicles at Ribbon Synapses

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Background: Sensory hair cells utilize specialized ribbon synapses to reliably transmit sensory information to the brain. Ribbon synapses have high rates of spontaneous vesicle release and function without fatigue. To sustain this level of release, a continuous supply of synaptic vesicles (SVs) must be trafficked to the presynapse. Recently we showed that Kif1a-mediated transport delivers new SVs along microtubules to the hair cell presynapse. Loss of Kif1a resulted in a depletion of SVs at the synapse, along with impaired neurotransmission. However, many other factors are needed to ensure that SVs are made, transported and recycled appropriately.

Methods: To study SV distributions, we study the zebrafish lateral-line system. We are using RNA-FISH to investigate expression of candidate genes involved in SV packaging, transport, and recycling. Then, to assess gene function we are using CRISPR-Cas9 to create mutations in candidate genes. In fixed hair cells, we use immunohistochemistry against SV markers including Vglut3, CSP, and Rab3a. To visualize SV populations in vivo, we use the vital dye LysoTracker. To assess hair-cell function, we assay for intact mechanotransduction using FM1-43 label, a dye that enters hair cells through mechanosensitive channels. To assess ribbon synapse function in our mutants, we use in vivo calcium imaging and loose-patch electrophysiology. To examine behavior in our mutants, we will use acoustic startle and rheotaxis assays to monitor lateral-line function.

Results: Currently we are using CRISPR-Cas9 to test the following candidate genes: Ap3, Rab3a/Madd, and Dynamin2, that we hypothesize are critical for SV biogenesis, transport, and endocytosis, respectively. RNA-FISH showed enrichment of Ap3m2, Rab3aa, Rab3ab, Madd, Dynamin2a, and Dynamin2b in zebrafish lateral-line hair cells. Preliminarily, we have found that ap3m2 mutants show less LysoTracker, Vglut3, and CSP label throughout the cell when compared to wild-type zebrafish hair cells, indicating Ap3m2 may be important to produce new vesicles. Additionally, ap3m2 CRISPR mutants show no significant difference in FM1-43 label throughout hair cells, indicating intact mechanotransduction. We are currently testing our other candidate genes using a similar approach.

Conclusions: Characterizing these mutants will allow us to shed light on the process of vesicle packaging, movement, and recycling in hair cells. This work will deepen our understanding of the multiple pathways that converge to supply SVs at ribbon synapses.

SA140. Decreased GABA: Maladaptive Breakdown or Compensatory Shift?

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Background: In humans and animals, decreased GABA in auditory cortex contributes to hyperexcitability, and may be maladaptive for speech recognition. If true, then modulating GABA may serve as a novel therapeutic target. We hypothesize that decreases in GABA may also contribute to the compensatory increases in cross-sensory plasticity that are observed in individuals with hearing loss (HL) and the finding that older adults rely on visual speech to understand speech-in-noise to a greater extent than younger adults. However, the age-related neurochemical (GABA) and microstructural (myelin) changes in the auditory system that mediate cross-sensory plasticity remain unknown. We hypothesized that aging and HL contribute to a decrease in GABA in auditory cortex, which is associated with conserved myelination (structural connectivity) between auditory and visual cortices, increasing cross-sensory plasticity and helping to preserve audiovisual (AV) speech recognition. If GABA is to serve as a potential therapeutic target, we need to first understand the extent to which the mechanisms of cross-sensory plasticity relate to previously reported maladaptive hyperexcitability in the auditory system that are also associated with lower levels of GABA.

Methods: Cortical evoked responses, +H-MRS, and Diffusion kurtosis imaging (DKI) were collected in 22 older and 7 younger adults. Cross-sensory activity was calculated as a ratio of cross-sensory activity (i.e., Visual evoked potentials (VEPs) in auditory cortex) relative to unimodal activity (VEPs in visual cortex). Granger causality was used to calculate functional connectivity between auditory and visual cortices. DTI tractography between auditory and visual cortices was used to examine the extent to which decreased GABA may contribute to conserved structural connectivity between auditory and visual cortices, increased cross-sensory plasticity, functional connectivity, and better AV speech recognition.

Results: Greater cross-sensory activity was observed in older adults compared to younger adults and was associated with decreased GABA in auditory cortex. Despite significant age-related decreases in total brain fractional anisotropy (FA), older adults did not exhibit significant age-group deficits in connections between visual and auditory cortices, and higher FA along this tract was associated with decreased GABA in auditory cortex. This relationship remained significant after accounting for whole brain measures of FA. GABA in auditory cortex was not associated with DTI metrics in other fiber tracts examined or whole brain measures. Measures of structural connectivity (FA) were positively associated with functional connectivity (Granger causality) between auditory and visual cortex ($r = 0.710$, $p = 0.001$). Greater cross-sensory activity was associated with better AV SIN.

Conclusions: We previously reported that decreased cortical GABA was maladaptive for auditory speech recognition. In contrast, these preliminary findings in older adults support the hypothesis that decreased GABA in auditory cortex may contribute to conserved myelination, enhancing connections between auditory and visual cortex.

SA141. Asymmetric Binaural Cue Reweighting in Unilateral Hearing Loss

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Background: Spatial hearing relies on the integration of interaural time (ITD) and level (ILD) differences, with perceptual weighting typically biased toward the more reliable cue.

Methods: To test how asymmetric hearing affects cue weighting, we measured the relative weighting of ITD and ILD cues in 48 adults: 12 within normal limits (WNL) of hearing sensitivity, 24 with simulated unilateral loss via earplug (SHL, left and right ear), and 12 with

chronic unilateral hearing loss (UHL, left and right ear). Participants completed a lateralization task with independently varying ITD and ILD cues. Lateralization slopes, bias, and model fit (R^2) were computed via multiple linear regression. ITD/ILD weight ratios were computed from the fit slopes.

Results: Lateralization slopes were significant for both ITD and ILD cues among WNL listeners ($p < .001$). SHL listeners showed significant but reduced ITD slopes ($p < .05$) and robust ILD slopes (indicating residual sensitivity to ILD and/or the corresponding monaural level cues). In UHL, ILD slopes remained significant, but ITD slopes were not ($p > .1$), indicating impaired timing cue use. Consequently, ITD/ILD weight ratios favored level cues more strongly in SHL ($M = 705 \mu\text{s/dB}$) and UHL ($M = 316 \mu\text{s/dB}$) vs. WNL ($M = 43.8 \mu\text{s/dB}$), with group differences confirmed for WNL vs. SHL and WNL vs. UHL (both $p = .004$).

WNL listeners showed robust fit quality (mean $R^2 = 0.72$) and minimal lateralization bias (-3%). In contrast, SHL produced degraded fits (mean $R^2 = 0.41$) and pronounced bias toward the unplugged ear (mean 46%). Chronic UHL showed intermediate fit quality (mean $R^2 = 0.53$) and reduced bias compared to SHL (mean 26%), although group differences in bias between SHL and UHL were not statistically significant (e.g., SHL-LE vs. UHL-LE: $t(8.11) = 2.22$, $p = .057$, $d = 1.16$; SHL-RE vs. UHL-RE: $t(6.24) = 2.10$, $p = .08$, $d = 1.15$).

Conclusions: These findings are consistent with the view that simulated UHL induces maladaptive, ear-specific reweighting, whereas chronic UHL reflects partial adaptation that reduces but does not fully resolve ear-specific biases, with implications for individualized rehabilitation strategies. [Supported by NIH R01-DC016643, P20-GM1090203, and Boys Town National Research Hospital]

SA142. Investigating the Perceptual Consequences of Low-Frequency Hearing Loss

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Background: Low-frequency sensorineural hearing loss (LFHL) is defined by elevated thresholds below ~ 2 kHz with preserved high-frequency sensitivity. This “reverse-slope” configuration differs from more common high-frequency sloping loss and presents unique challenges for diagnosis and treatment. “Off-frequency listening” can lead to overestimation of apical function on the audiogram and underestimation of perceptual deficits. Amplification within a low-frequency dead region may lead to masking of remaining high-frequency cues, without contributing additional information. The present study aims to explore the perceptual consequences of LFHL, with the long-term goal of providing better diagnostics and more targeted treatment to this overlooked population.

Methods: A battery of behavioral tests were designed to probe specific consequences of LFHL, including identifying potential cochlear dead regions and relating the psychoacoustic outcomes to measures of speech intelligibility. Tests included: Psychophysical tuning curves, designed to identify dead regions through shifts in PTC tip frequency; amplitude and frequency modulation detection, designed to identify deficits in temporal envelope and/or fine-structure processing; pitch discrimination with pure and harmonic complex tones, designed to identify deficits in the processing of spectrally resolved and/or unresolved harmonics; and speech perception in noise with auditory-only and audio-visual speech in broadband as well as lowpass- and high pass-filtered conditions.

Results: Preliminary results from individual participants indicate idiosyncratic outcomes that will be related to theories of underlying mechanisms, including dysfunction of inner and/or outer hair cells. Once complete the findings will provide a systematic study in this previously understudied population.

Conclusions: This project will provide a systematic characterization of the perceptual consequences of LFHL, clarifying how listeners exploit available cues when low-frequency place coding is disrupted. Findings will refine theories of pitch and auditory coding, support better classification of LFHL, and inform the development of an abbreviated diagnostic battery to guide perceptually appropriate management and treatment strategies.

SA143. At-Home Augmented Reality Training Improves Speech-In-Noise Performance in Older Adults

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Background: Age-related hearing decline affects speech comprehension in noisy environments and sound localization abilities, critical for daily communication and safety. While our previous augmented reality (AR) auditory training showed promise in younger adults, implementation in at-risk populations and clinical populations remains unexplored. This study evaluates a home-based AR auditory training system in adults aged 50-60 and introduces its expansion to cochlear implant (CI) users.

Methods: Eleven adults ($M=53\pm3$) with normal hearing completed home-based AR auditory training using our smartphone application featuring 6DoF audio rendering. Participants completed four training sessions and one evaluation session weekly for four weeks, averaging 265 minutes total training time. The gamified training presented two distinct tones in spatial noise, requiring selective attention and localization. Pre/post assessments included the Korean Matrix Sentence Test (KMST) at 0dB, -6dB, and -9dB SNR. Weekly in-game evaluation sessions evaluated training efficacy and performance improvements without feedback confounds. Reliable Change Index (RCI) analysis distinguished true training effects from test-retest variability, while System Usability Scale (SUS) scores evaluated the learnability and ease-of-use of the AR system for this age group. Qualitative user feedback was obtained from exit interviews after the training period. Building on these normal-hearing results, we initiated feasibility testing with CI users to evaluate clinical translation potential.

Results: KMST performance showed significant improvements, with 6/11 participants (55%) demonstrating reliable improvement at -6dB SNR based on RCI criteria (threshold $\geq 9.18\%$), with mean improvement of 10.09% ($SD=6.27$). At -9dB SNR, participants improved by 8.55% ($SD=6.53$), and at 0dB SNR by 3.00% ($SD=2.90$). Importantly, improvements in sound localization accuracy during training sessions significantly correlated with KMST gains at -6dB SNR ($\rho = 0.639$, $p = 0.034$), suggesting shared underlying mechanisms between spatial hearing and speech-in-noise processing. The SUS score of 70.2 validates that older adults can adopt AR technology for training, though the interface and instruction require refinement. User feedback revealed that while training difficulty was appropriate, current gamification elements were insufficient for sustained engagement. Pilot testing with CI users showed promising engagement when training difficulty was adjusted and system hardware accommodated implant requirements.

Conclusions: Home-based AR auditory training improved speech-in-noise comprehension, with over half of participants showing gains beyond test-retest variability at challenging SNRs based on RCI analysis. The significant correlation between in-game localization improvements and speech-in-noise gains suggests that spatial hearing training may enhance speech processing through shared attentional mechanisms. Notably, the usability scores demonstrate that AR technology can be successfully deployed for home-based auditory rehabilitation with minimal guidance. However, maintaining user engagement throughout the 4-week protocol proved challenging, indicating the need for improved gamification strategies. The successful adaptation for CI users represents a critical step toward digital therapeutic implementation. Future work will expand the CI cohort, and enhance engagement mechanisms for sustainable training.

SA144. Hyperbaric Oxygen Therapy for Sudden Sensorineural Hearing Loss

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Background: Idiopathic sudden sensorineural hearing loss (ISSNHL) is a rapid decrease in hearing ability, typically occurring in one ear. Treatments for ISSNHL are difficult to study as patients can recover spontaneously, making it difficult to separate treatment effects from the natural course of the disease. Current first-line therapies are oral and intratympanic corticosteroids, yet the mechanisms underlying their benefit remain poorly understood. Hyperbaric oxygen therapy (HBOT) has also emerged as a potential ISSNHL treatment, yet it is not clear if it offers benefits beyond steroids. This study assessed the effect of HBOT, in combination with corticosteroids, on hearing improvement following ISSNHL.

Methods: We analyzed data from the HBOT Registry, a global, multicenter collaboration that tracks outcomes of SHL patients treated with HBOT across 33 institutions. Hearing thresholds (pure tone average, PTA, of 0.5, 1.0, 2.0 and 4.0 kHz) were assessed before and after HBOT initiation, with clinically significant recovery defined as ≥ 15 dB improvement in PTA or return to baseline. Patients in both datasets received oral and/or intratympanic corticosteroids prior to HBOT. In addition, we conducted a retrospective case series of ISSNHL patients at Dartmouth-Hitchcock Medical Center, who received HBOT following corticosteroid therapy, to determine if there were cases that showed a clear benefit beyond steroids. Inclusion criteria included documented ISSNHL, prior corticosteroid treatment, and completion of at least 10 HBOT sessions within 14 days of symptom onset.

Results: HBOT Registry data showed that HBOT was associated with overall improvement in measured PTAs among ISSNHL patients (n=256). Specifically, 41.02% of patients were found to have achieved clinically significant hearing recovery. The overall mean improvement in PTA following HBOT, averaged across all patients, was 14.37dB HL. Since most patients typically receive steroids prior to HBOT, this suggests an additional benefit from HBOT. In the Dartmouth-Hitchcock case series, 10 cases were highlighted where HBOT was associated with an additional hearing recovery beyond what the patient had obtained with steroids. This recovery was particularly apparent amongst low frequencies (i.e., 0.5-1.0 kHz). This is a limited case series and additional study on the interaction between corticosteroid therapy and HBOT is needed.

Conclusions: Our findings suggest HBOT improves hearing beyond what is achieved with corticosteroids in some individuals. Incorporating HBOT into treatment algorithms may

improve outcomes in refractory cases, though prospective, controlled studies are needed to guide clinical decision-making and patient selection.

SA145. Stratifying Complex Sensorineural Hearing Loss Using Non-Invasive Biomarkers: A Replication Study

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Background: Sensorineural hearing loss (SNHL) is a complex disorder, often the result of several etiologies accumulated across the lifespan, resulting in variable treatment outcomes. Current audiometric testing cannot distinguish different inner ear pathologies that may contribute to the umbrella diagnostic category of SNHL. As a result, clinical management focuses on audibility and patient reports rather than each patient's cochlear pathophysiology profile. Furthermore, different cochlear pathologies such as damage to cochlear sensory cells (OHCs and IHCs), afferent synapses, or degeneration of the stria, can affect listening outcomes for speech in divergent ways. Recent cross-species studies (Hauser, 2025) suggest that a non-invasive multi-biomarker battery may allow for the stratification of different cochlear pathologies in SNHL and predict listening outcomes. Here, we aim to replicate a portion of these studies by testing whether this multi-biomarker battery can better predict speech-in-noise scores, compared to the audiogram alone.

Methods: Participants aged 18–70 years were recruited across the continuum of normal hearing and symmetric moderate SNHL from a University of Pittsburgh research participant database (N=32 so far). Participants completed air- and bone-conduction audiometry (0.25–16kHz AC, 0.5–4kHz BC), wideband tympanometry, and provided demographic, otologic, and audiology surveys to mirror a clinical evaluation. Then, a battery of non-invasive biomarkers, including envelope following responses to rectangularly amplitude-modulated tones (RAM EFRs) recorded with a 32-channel EEG cap, wideband middle ear muscle reflexes (WB-MEMR), and swept-tone distortion-product otoacoustic emissions (DPOAEs) were acquired on each participant based on pre-clinical results from Hauser (2025). To measure suprathreshold listening outcomes, spectrotemporal modulation thresholds, and speech-in-noise scores were measured across two tasks emphasizing energetic masking and higher cognitive factors, respectively.

Results: Preliminary data are consistent with the notion that the multi-biomarker battery can capture aspects of SNHL pathophysiology that are not discernable from the audiogram. Specifically, after controlling for residual age or cognitive differences, the biomarker battery predicted individual variations in listening performance beyond those predicted by the audiogram.

Conclusions: Our preliminary findings highlight variability in suprathreshold listening abilities that are not fully explained by audiometric thresholds in overt SNHL. Further data collection is ongoing and will supplement the data collected by Hauser (2025) to evaluate the potential of a non-invasive multi-biomarker battery approach for precision diagnostics in audiology and predicting listening outcomes.

SA146. Presynaptic Regulation of Quantal Size at Calyx of Held Synapses Following Conductive Hearing Loss

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Background: In response to decreased sensory input with hearing loss, the central nervous system undergoes compensatory changes. These changes are associated with neuronal activity alteration and map reorganization, as well as modifications in synaptic function.

Methods: Using the mouse calyx of Held, a giant glutamatergic terminal in medial nucleus of the trapezoid body (MNTB) of the auditory brainstem, we examined the synaptic function changes following unilateral conductive hearing loss (earplugging).

Results: Our results showed that the amplitude and frequency of miniature excitatory postsynaptic currents (mEPSCs) increased in hearing loss MNTB compared to the normal side after 2-3 days of earplugging. We also estimated the synaptic properties changes using a high-frequency stimulation protocol, showing an increased release probability upon hearing loss. Lastly, bath application of a low-affinity, rapidly dissociating AMPA receptor antagonist γ -DGG revealed that higher concentration of glutamate is packed into the vesicles of hearing loss calyces.

Conclusions: In conclusion, our results showed that hearing loss induces presynaptic homeostatic plasticity by increasing vesicular glutamate concentration and neurotransmitter release probability.

SA147. Changes in Local and Global Neural Dynamics Across Brain States in Congenitally Deaf Mice

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Background: Hearing loss may induce global cortical changes beyond local effects on the auditory cortex. These changes may underlie the increased dementia risk and cognitive decline associated with hearing loss. However, the extent of these cortical network changes beyond the auditory system remains unclear.

Methods: To study these changes we combined intracortical multielectrode array recordings and 30-channel topographic EEG in congenitally deaf Otof (deaf5Jcs/Kjn) mice. We compared local changes in the deaf auditory cortex to those in the neighboring somatosensory cortex, as well as to distributed brain-wide global changes in EEG activity. To assess the dynamics of ongoing activity we examined cortical activity during controlled transitions between anesthesia-induced brain states. We then quantified periodic and aperiodic changes in spectral activity, coherence, and connectivity measures between regions.

Results: Deaf mice showed a clear local decrease in gamma and delta activity in the deaf auditory cortex. Importantly, the neighboring somatosensory cortex was unaffected, indicating a localized effect. At the global EEG level, we found a change of gamma activity in occipital regions; however, activity in frontal areas remained similar in hearing and deaf mice. Further, although coherence between cortical areas changed across brain states, there were no consistent differences in coherence between hearing and deaf mice. Effects on functional connectivity were weak and heterogeneous.

Conclusions: The results show that congenital deafness leads to consistent local effects in the auditory cortex. In contrast, the effects on global EEG activity appear weaker and are state-

dependent. The results suggest that brain-wide consequences of hearing loss are subtle and may only be detectable under certain conditions. This underscores the importance of examining neural dynamics across different brain states.

SA148. Mitochondrial Localization of Androgen Receptor in the Organ of Corti: Evidence From Super-Resolution Confocal Microscopy

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Background: Sex is a biological factor that influences inner ear homeostasis and disease susceptibility. To investigate the molecular basis of sex differences, we previously examined cochlear gene expression and identified a group of sex-biased genes in the mouse cochlea. Functional analysis revealed that male-biased genes are associated with mitochondrial energy production. Because male hormones are major regulators of male traits, we hypothesized that they contribute to the regulation of male-biased mitochondrial function genes. The androgen receptor (AR), the primary mediator of male hormone action, has been detected in the cochlea. This study was designed to characterize the subcellular distribution of AR in the cochlea, with particular attention to its localization in mitochondria. In addition, we used bioinformatic tools to determine which male-biased mitochondrial function genes have been previously linked to AR.

Methods: Young male and female CBA/CaJ and C57BL/6J mice were used in this study. To examine the subcellular distribution of AR protein, cochleae were dissected to isolate the sensory epithelium. The tissues were processed for immunohistology to assess AR expression in the organ of Corti using confocal microscopy. Colocalization of AR expression with mitochondria, identified by the mitochondrial marker protein Tomm20, was determined using super-resolution microscopy. The acquired images were analyzed using the Imaris image-processing software. To investigate a potential link between AR and male-biased mitochondrial function genes, the web-based transcription factor database TFlink was used to retrieve known AR-responsive genes. This gene list was compared with male-biased genes to identify overlaps, which were then analyzed for functional associations using the Database for Annotation, Visualization, and Integrated Discovery (DAVID).

Results: In the organ of Corti, AR expression was detected primarily in Deiters' cells, with the strongest signal in the superior portion of the cell body near the Deiters' cell cup. Weak immunostaining was also observed in other supporting cell types, including pillar cells and Hensen's cells. The staining displayed a tonotopic gradient, weaker in the apical turn and stronger in the middle and basal turns. AR immunoreactivity was confined to the cytoplasm, showing a granular distribution, while nuclei lacked detectable staining. To determine its subcellular localization, tissues were double-stained with the mitochondrial marker protein Tomm20. Super-resolution microscopy confirmed that AR expression was localized to mitochondria. Bioinformatic analysis revealed that many male-biased mitochondrial function genes are AR-responsive, suggesting that AR may contribute to the male-biased expression of genes associated with mitochondrial energy production.

Conclusions: The study shows that AR is present in the mitochondria of cochlear supporting cells and that many male-biased mitochondrial function genes are AR-responsive. These results suggest that AR may contribute to sex differences in mitochondrial gene activity and energy production in the cochlea.

SA149. Regulation of Mitochondrial Genes by Female Hormones in the Mouse Organ of Corti

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Background: Mitochondria are energy-producing organelles essential for ATP generation, redox balance, and cell signaling. Our previous studies identified sex differences in the expression of mitochondrial function-related genes between males and females, suggesting that sex hormones may influence mitochondrial gene expression. This study examined how female hormones regulate mitochondrial DNA (mtDNA) copy number in the female cochlea across the estrous cycle. Because hair cells and adjacent supporting cells in the organ of Corti are rich in mitochondria, we also assessed the expression of mitochondrial function-associated genes in this tissue across the estrous cycle.

Methods: Young CBA/CaJ mice were used. Female mice were divided into two groups, proestrus or diestrus, based on the assessment of vaginal cytology. Mice were sacrificed, and the cochleae were collected at these two phases. Whole-cochlea tissues containing the lateral wall, sensory epithelium, and osseous spiral lamina were used to assess mtDNA copy number. In addition, we isolated the organ of Corti tissue enriched for sensory cells and nearby supporting cells using our unique microdissection technique. Total RNA was extracted to quantify mRNA levels of three mitochondrial genes (ND1, ATP8, CYTB) involved in mitochondrial energy production.

Results: Relative mtDNA copy number was determined by calculating the ratio of mtDNA to nuclear DNA and comparing the values between the proestrus and diestrus stages of the estrous cycle. mtDNA copy number was significantly higher in proestrus than in diestrus (unpaired Student's t-test, $p = 0.0142$), suggesting increased mitochondrial abundance and biogenesis. For gene expression analysis, three mitochondrial genes that exhibited sex-biased expression in our previous study were selected. ND1 encodes a subunit of Complex I, ATP8 encodes a subunit of ATP synthase (Complex V), and CYTB encodes a component of Complex III. Slc26a5 (a sensory-cell marker) was used as the reference gene to normalize the expression levels of ND1, ATP8, and CYTB. The analysis revealed that all three genes showed higher expression in proestrus than in diestrus (ND1: 4.86 ± 2.29 fold, $p = 0.0023$; ATP8: 4.97 ± 2.39 fold, $p = 0.0024$; CYTB: 4.75 ± 2.42 fold, $p = 0.0024$). This expression enhancement during proestrus is consistent with the increased mitochondrial DNA copy number observed in this phase of the estrous cycle.

Conclusions: The combined increase in mtDNA copy number and elevated expression of ND1, ATP8, and CYTB during proestrus suggests a phase-specific enhancement of mitochondrial function across the estrous cycle. Such hormone-linked bioenergetic variation may contribute to cycle-related differences in cochlear physiology and vulnerability to stressors.

SA150. Assessment of the Structural and Molecular Integrity of Human Organ Donor Inner Ear Specimens

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Background: Studies of the human inner ear are limited by the difficulty of obtaining high-quality tissue specimens for cellular-level analysis. Traditional post-mortem processing of temporal bones requires delayed time to fixation and prolonged decalcification, which can compromise the molecular integrity of tissue. Developing methods to rapidly harvest inner ear organs from human organ donors while maintaining their structural and molecular fidelity would enable advanced analyses that could provide critical insights into the pathophysiology of hearing and balance disorders as well as the molecular architecture of the normal inner ear.

Methods: Using a novel technique for harvesting human temporal bones from organ donors, inner ear organs were fixed in 10% formalin and dissected using a surgical drill and microsurgical instruments. A brief 24-hour decalcification in 5% EDTA was performed to remove residual bone and otoconia. To assess the structural integrity and expression of proteins commonly used in non-human models, whole mount cochlear and vestibular end-organs were incubated overnight with primary antibodies, followed by incubation with Alexa fluorophore-conjugated secondary antibodies. Secondary antibody solutions were supplemented with the nuclear dye DAPI and Alexa 405 fluorophore-conjugated phalloidin. After several washes, specimens were mounted for confocal microscopy imaging and analysis.

Results: All vestibular end-organs (utricle, saccule, and three cristae) as well as the cochlea were successfully harvested structurally intact. Immunostaining demonstrated preservation of hair cells with stereocilia bundles, supporting cells, spiral ganglion neurons, and afferent and efferent innervation. Confocal imaging confirmed cellular morphology and marker expression comparable to that observed in established animal models.

Conclusions: Harvesting inner ear organs from organ donors within ~1-2 hours postmortem allows structural and molecular preservation of the sensory tissues in their entirety with rapid fixation and a considerably shortened decalcification period. Maintaining the molecular integrity of these specimens is crucial, thus enabling future multi-omic analyses aimed at further characterizing both normal and diseased human inner ear structures.

SA151. Advancing Imaging Approaches for 3D Characterization of Inner Ear Cell Types Across Development

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Background: The inner ear is a complex and specialized structure essential for both auditory and vestibular capabilities. It is enclosed inside a very hard bone, making it difficult to access with imaging techniques that are non-invasive or preserve its 3-dimensional structure. Over postnatal development, the mouse inner ear undergoes significant structural and cellular changes that are crucial for its functional maturation. We employed two imaging techniques, magnetic resonance imaging (MRI) paired with micro-computed tomography (uCT), and whole tissue clearing to analyze the structural and cellular morphology of the developing mouse inner ear.

Methods: In vivo mouse skulls from animals ages p1 to adulthood were imaged using MRI followed by uCT. MRI provided visualization of soft tissue, while uCT enabled visualization of bony structures. For whole tissue clearing, temporal bones were first immunostained for markers of various cell types, including hair cells, endothelial cells, and spiral ganglion neurons, then cleared. Samples were imaged using lightsheet microscopy, generating 3D

reconstructions used for quantitative and morphological analysis across developmental stages.

Results: MRI and uCT imaging enabled volumetric quantification of inner ear structures, including the cochlea, vestibular organs, and endolymphatic sac. These volumetric measurements revealed developmental changes to size and morphology of structures over time. Similarly, antibody stained and cleared temporal bones enabled quantification of different cell types and morphologies at different age points within the whole bony labyrinth structure.

Conclusions: This imaging toolkit will be critical for studying the postnatal development of the inner ear. When applied to models of hearing loss, these tools will be useful for revealing developmental emergence of morphological abnormalities, as well as evaluating the efficacy of potential therapeutic interventions.

SA152. Including Pressure Focusing in Time-Domain Transmission-Line Cochlear Models

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Background: One-dimensional transmission-line (TL) models are the simplest cochlear models that include an explicit description of the traveling wave. TL models are computationally light, making them suitable for time-domain nonlinear simulations of cochlear mechanical responses. Compared with simpler, faster filter-bank models, TL models can reproduce phenomena that arise from wave propagation—such as otoacoustic emissions and longitudinal coupling of cochlear filters. In recent years they have been employed in practical applications, for example to train AI models that improve sound-processing strategies in hearing aids.

However, TL models struggle to replicate several cochlear nonlinear phenomena, such as the full extent of the nonlinear dynamic range and “high-side” suppression. These shortcomings stem from ignoring the important hydrodynamic pressure-focusing effect, which boosts basilar-membrane (BM) vibration near the characteristic frequency (CF) and naturally emerges in 2-D and 3-D models of cochlear mechanics.

Methods: The pressure-focusing effect in 2-D and 3-D models can be quantified as the ratio between the pressure that actually drives the BM and the average pressure in the scala; this ratio (α) is a function of wavelength and cochlear dimensions. TL models assume that the average and driving pressures are identical and currently compensate for the missing pressure-focusing by making the BM oscillators unrealistically sharply tuned.

By theoretically analysing the behaviour of 2-D and 3-D models, we derived an approximation that expresses the pressure-focusing factor as a rational function of wavelength, whose parameters depend on cochlear geometry. Mathematically this is equivalent to a spatial filter that converts average pressure into driving pressure. The spatial filter can be incorporated into a time-domain TL model, yielding a digital analogue of pressure focusing.

Results: Theoretical results based on WKB solutions of TL and 3-D models suggest that our “pressure-focusing” trick markedly improves TL-model performance—e.g., by expanding the nonlinear dynamic range—so that TL models can operate in all practical respects much like full 3-D models. We implemented the strategy in a state-space solver for a TL model,

demonstrating that it can be added computationally efficiently to a generic cochlear-mechanics model. So far we have tested the time-domain implementation in a passive, linear model.

Conclusions: Our work shows that wavelength- and dimension-dependent pressure focusing, inherent to the 3-D cochlea, can be incorporated into 1-D TL models. This inclusion is expected to substantially improve the performance and quality of TL models. Preliminary results indicate that adding the pressure-focusing term is straightforward in time-domain simulations, especially when using a state-space formalism.

SA153. Tonic Outer Hair Cell Motility in Mice With Little Other Evidence of Active Cochlear Mechanics

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Background: In response to sound, cochlear outer hair cells (OHCs) undergo both tonic and phasic length changes. These length changes presumably reflect the OHCs' electromotile response to tonic and phasic changes in membrane potential that are driven by the mechanotransduction process. Recently, tonic length changes have been observed in mice with stereociliary defects that lead to varying amounts of phasic OHC activity and the near-total loss of cochlear amplification. These mice include salsa mice, which progressively lose the stereociliary tip links, and Triobp(Δ ex8/ Δ ex8) mice, in which the stereocilia lack rootlets and are prone to damage and degeneration. Though the tonic responses in these mice remain of unclear origin, they could be attributed to the presence of residual mechanotransduction currents that are small but highly asymmetric. If so, the tonic responses should depend on the overall integrity and mechanotransduction capability of the stereociliary bundle. This was addressed here by examining tonic OHC motility in salsa/Triobp(Δ ex8/ Δ ex8) double-homozygous mice, in which stereocilia function is likely to be more impaired than in either of the individual strains.

Methods: Optical coherence tomography was used to image and measure sound-evoked displacements from the cochlear apex in 3- to 4-week-old salsa/Triobp(Δ ex8/ Δ ex8) mice. Mice were first deeply anesthetized, placed on a heating pad, and fixed to a head holder. The left middle ear space was then surgically accessed and a probe used to deliver sound stimuli was sealed over the resected ear canal.

Results: Tone-evoked displacements of the basilar membrane, OHC region, and tectorial membrane exhibited little to no evidence of active amplification or phasic OHC motility in salsa/Triobp(Δ ex8/ Δ ex8) mice. Distortion-product otoacoustic emissions were also essentially absent. Nevertheless, tonic displacements consistent with OHC contractions were elicited in roughly one-third of the mice tested. While the fraction of mice with detectable tonic OHC motility was lower than what was previously found for salsa and Triobp(Δ ex8/ Δ ex8) mice at similar ages, the tonic displacements that were observed in salsa/Triobp(Δ ex8/ Δ ex8) mice could be as large as those recorded in the individual strains. Endolymphatic hydrops was seen in several mice though did not appear to be correlated with the presence or absence of tonic responses.

Conclusions: The lower prevalence of tonic OHC responses in salsa/Triobp(Δ ex8/ Δ ex8) double-homozygous mice compared to in salsa and Triobp(Δ ex8/ Δ ex8) mice suggests that the responses likely depend on stereociliary integrity and residual mechanotransduction currents. Nevertheless, the observation of large tonic responses in mice with no other

evidence of active, nonlinear cochlear mechanics further highlights how tonic and phasic OHC motility can appear dissociated in certain cases of hearing impairment. The mechanisms that drive and shape the tonic responses in such cases remain to be fully determined.

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SA154. Phase or Fade: The Organ of Corti's Internal Phase-Gain Correlation

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Background: A playground swing rises only when the push arrives in phase with its motion; a mistimed push slows it. The cochlea is likewise a feedback device whose effectiveness hinges on relative timing among elements of the organ of Corti (OoC)—notably the basilar membrane (BM), reticular lamina (RL), and tectorial membrane (TM). In the intact ear, however, a traveling wave sweeps along the partition, accumulating phase and redistributing amplitude. Motion at any single place therefore blends local micromechanics with global propagation, obscuring the lead-lag relations that govern whether the system adds or removes energy. We therefore set out to examine internal phase under conditions that emphasize the local behavior of the OoC.

Methods: We studied stabilized ex vivo gerbil cochlear segments from the middle turn. Each segment was mounted in a sealed two-compartment chamber that preserved the native ionic separation, maintained physiological temperature, and allowed us to impose and monitor a near-physiological endocochlear potential (EP). Acoustic stimuli were delivered to the preparation while we made phase-resolved optical measurements of motion across distinct OoC substructures (e.g., BM, RL, TM). The nature of the preparation ensured the absence of traveling-wave contributions in our recordings.

Results: With the EP present, the organ of Corti exhibits an organized pattern of internal phase relations among its substructures near the local best frequency; when the EP is removed, this pattern disappears in the same preparation. This qualitative dependence on EP is consistent with in-vivo observations that altering the electrochemical drive reorganizes micromechanics.

Conclusions: As with a swing, timing makes gain. Under physiological drive, the OoC settles into an organized phase relationship; remove the drive and that organization collapses. By suppressing traveling-wave effects, the segment approach isolates local timing, clarifying how internal phase is arranged when the system is powered.

SA155. Does an Interaction Between the Estrous Cycle and TRPA1 Signaling Influence Cochlear Responses?

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Background: Transient receptor potential ankyrin 1 (TRPA1) channels are highly expressed in nociceptive neurons and in Hensen's cells of the cochlea. Specifically, TRPA1 gating in Hensen's cells results in prolonged calcium responses across the organ of Corti causing long-lasting tissue displacements. We previously showed that TRPA1 activity is as a component of

the noise-induced temporary threshold shift (TTS) (Velez-Ortega et al., 2023, Nat Commun) and that five days after noise exposure, TRPA1-deficient mice experience a significant reduction in the amplitude of cochlear microphonics measured remotely through scalp electrodes (rCM) (Radomski et al., 2025, Hear Res). Interestingly, our unpublished preliminary data indicate that (a) young TRPA-deficient female mice (5-6 weeks old) have larger auditory nerve activity than both TRPA1-deficient males and wild-type littermates and that (b) wild-type female mice exhibit larger variability in the amplitude of rCM recordings after noise exposure than wild-type male mice. Of note, TRPA1 binds promiscuously to a multitude of ligands including estrogen metabolites (Xie et al., 2022, JCI Insight). Here, we hypothesize that cochlear responses are affected by an interplay between estrogen and TRPA1.

Methods: We utilize anesthetized TRPA1-deficient mice (Kwan et al., 2006, Neuron) and wild-type littermates maintained in a C57Bl/6 background. We record auditory brainstem responses (ABR) to click stimuli as well as rCM responses to an 8 kHz tone burst, with alternating 0° and 180° phases interleaved. In female mice, vaginal swabs are collected daily and hearing testing is performed at designated phases of the estrous cycle.

Results: In young mice (3-6 weeks old), ABR wave I amplitude to loud sound stimuli (> 90 dB SPL) is greater in TRPA1-deficient female than wild-type female mice or male mice of either genotype. As the mice age (4-5 months old); however, TRPA1-deficient male mice also exhibit larger ABR wave I amplitudes to high sound stimulation in comparison to wild-type male littermates. Based on these newly-identified sex differences, we reanalyzed our previously-published data linking TRPA1 activity to TTS and indeed found sex differences in the magnitude of some cochlear responses in the TRPA1-deficient mice. Our recordings of ABR and rCM at specific phases of the estrous cycle are currently undergoing.

Conclusions: Our data indicate a potential interplay between biological sex and TRPA1 signaling that might affect cochlear responses before and after noise exposure.

SA156. Cochlear Vibratory Responses to Voiceless Fricatives

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Background: Although human speech can contain spectral energy up to 20 kHz, and listeners can perceive sounds up to 15–20 kHz, much research on speech perception has focused on frequencies below 5 kHz. This reflects the longstanding assumption that high-frequency energy (HFE, > 5 kHz) is unnecessary for intelligibility, based on early views that speech-relevant frequencies end near 4 kHz. Recent evidence, however, indicates that HFE—particularly in fricatives—can enhance clarity, localization, and robustness in noise. The mechanical and neural encoding of these components in the inner ear remains poorly understood. Addressing this gap is important both scientifically and for improving hearing aids. We therefore measured cochlear vibratory responses to fricative consonants in the mouse.

Methods: Vibratory responses were recorded in vivo from intact cochleae of mice (P28-P84) using optical coherence tomography. Measurements were obtained from basilar membrane (BM) sites with characteristic frequencies near 8–10 kHz. Acoustic stimulation included tones across frequency and intensity ranges, as well as two words (“say” and “cease”) spoken by a female native speaker of American English, presented at several intensity levels.

Middle-ear motion was also measured in the same animals. BM response amplitudes were derived using Fourier and short-time Fourier (spectrogram) analyses. Waveform envelopes were estimated via the analytic signal representation.

Results: Nonlinear cochlear filtering of the fricative /s/ was evident from level-dependent spectral changes in BM responses. Both the stimuli and BM responses to /s/ contained spectral energy exclusively above ~4 kHz. While the speech stimuli extended to 15–20 kHz, BM responses were limited to ~10 kHz for softer levels and ~6–7 kHz for the loudest levels, reflecting cochlear filtering nonlinearities. Despite this filtering, BM vibratory responses remained intelligible when resynthesized as sound, regardless of stimulus level. Moreover, the envelopes of BM responses to /s/ closely matched stimulus envelopes, even at the highest intensities.

Conclusions: We present the first direct measurements of cochlear mechanical vibrations evoked by speech sounds. Our findings demonstrate that fricatives with HFE drive nonlinear but intelligible BM responses in the mouse cochlea, with envelopes preserved across levels. These results highlight how the cochlea encodes speech components above 5 kHz, providing new insight into the peripheral basis of speech perception.

SA157. Phase-Sensitive Optical Coherence Elastography for Characterizing Cochlear Micromechanics With Multiple Excitation Methods

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Background: Optical coherence tomographic (OCT) vibrometry has been successfully applied for measuring sound-induced nanoscale displacements of inner-ear structures, providing important insights into cochlear dynamics. However, it is limited to measure vibrational responses. Phase-sensitive Optical Coherence Elastography (OCE) extends these capabilities by using OCT phase information to measure displacement fields and their spatial and temporal gradients, such as strain and strain rate. Relating these gradients to applied loads or wave-propagation behavior enables estimation of local mechanical properties. This allows non-invasive, non-contact characterization of both surface and sub-surface mechanical behavior of intracochlear structures, advancing our understanding of the cochlear mechanics and supporting improved biomechanical models of hearing.

Methods: We adapted a commercial spectral-domain high-speed OCT system (Thorlabs Telesto, 1300 nm center wavelength, 146 kHz A-scan rate) for elastography and developed custom software for external triggering, acquisition control, and phase-resolved displacement analysis. The methodology for motion and mechanical property measurements was validated in phantom samples by comparison with independent techniques, including Laser Doppler Vibrometry (LDV) and nanoindentation. Experiments on cadaveric human cochleae were conducted non-invasively through the round window membrane (RWM), providing access to internal cochlear structures without disturbing the partition. Excitation methods included acoustic clicks, tones, and point-source pulsed laser stimulation. Pressure sensors positioned near the RWM characterized distributed acoustic loads applied to the cochlea, while laser-induced localized loading of cochlear tissue was calibrated photoacoustically. Nanometer-

scale displacements were extracted from OCT phase signals. The acquisition framework employed phase-locked detection with repeated M-scans at individual points, which were reconstructed as time-resolved B-scans to capture spatiotemporal motion patterns. These data were further analyzed to estimate localized mechanical properties. Computational approaches, including finite element modeling and localized surface and sub-surface wave estimation, were evaluated within this framework.

Results: The adapted OCE system detects nanometer-scale cochlear motions with high fidelity across multiple excitation methods. The accuracy of these motion measurements has been validated against LDV on artificial samples. Elastography results in phantom materials are being compared with independent nanoindenter measurements, and measurements are being extended to cochlear samples. Once completed, elastograms are expected to reveal spatial variations within the cochlear partition that correlate with anatomical structures, supporting the feasibility of assessing cochlear tissue mechanical properties such as elasticity, stiffness, and damping.

Conclusions: Phase-sensitive OCE, with phase-locked detection and multi-modal excitation, extends beyond vibrometry by enabling direct, non-invasive, and non-contact estimation of cochlear micromechanical properties at multiple sites across the cochlear partition. The ongoing work demonstrates the feasibility of combining high-speed OCT, custom elastography software, frequency-domain analyses, and computational modeling to characterize cochlear micromechanics. Continued development is expected to further establish OCE as a reliable tool for the hearing sciences.

SA158. Full-Field Evaluation of Middle Ear Nonlinearities Across Moderate and High-Intensity Sound Levels by High-Speed 3D-Digital Image Correlation and Laser Doppler Vibrometry

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Background: At everyday sound levels, the middle ear responds in a proportional way: doubling input pressure doubles motion of the tympanic membrane (TM) and ossicles. At intense levels, nonlinearities emerge and this proportionality no longer holds, altering sound transmission to the inner ear. Such behavior may result from large TM deformations (geometric nonlinearity), tissue effects (material nonlinearity), or changes in ossicular coupling. Understanding when nonlinearities appear and what mechanisms drive them is important for biomechanical modeling, hearing protection, surgical graft development, and defining injury thresholds relevant to blast trauma.

Methods: Previously, we validated our methodology in a single cadaveric temporal bone (TB). Since then, we optimized the technique with improved pressure calibration and a dedicated processing pipeline. Here, three TBs with intact middle ears were tested under progressively increasing excitations, from loudspeaker-generated acoustic clicks (50–2000 Pa, ~128–160 dB SPL) to shock-tube overpressures (500 Pa–20 kPa, ~148–180 dB SPL). The external ear canal was removed to expose the TM for high-speed three-dimensional Digital Image Correlation (3D-DIC, 50,000 fps). Input pressures were recorded with calibrated broadband sensors, while TM displacements were measured by high-speed 3D-DIC. Laser

Doppler Vibrometry (LDV) measured umbo and stapes motions for validation and ossicular transmission analysis. TM surface preparations were tested to optimize optical contrast while minimizing alteration. Custom software enabled synchronization of pressure and displacement, filtering, noise-floor estimation, and computation of transfer functions (TFs) and impulse responses (IRs). Full-field TM motion combined with pressure data enabled energy coupling analyses, where the ratio of TM volume displacement to cumulative pressure energy flux was calculated as an additional metric of nonlinear responses.

Results: Reliable TFs and IRs were reconstructed across all TBs under multiple excitations. At low-to-moderate levels, responses were linear, consistent across samples, and in agreement with published data. At higher pressures, evidence of nonlinear behavior appeared. TFs showed compressive growth, with displacements increasing less than proportionally with pressure, and broadened frequency peaks, suggesting greater damping. IRs exhibited stable onset timing but demonstrated amplitude compression and faster decay at higher intensities. Umbo and stapes analysis indicated changes in transmission with increasing load, suggesting ossicular coupling contributes to the nonlinear behavior. Full-field analyses further showed the ratio of TM volume displacement to cumulative pressure energy flux decreased with increasing pressure, supporting nonlinear processes.

Conclusions: This study showed the middle ear nonlinearity at high intensities can be described by compressive growth, broadened frequency peaks, changes in decay, and altered ossicular coupling, which affect middle ear sound transmission. Future work will refine experimental protocols and use the datasets to develop constitutive models of middle ear tissues, which will be implemented and validated in a nonlinear finite element framework to better characterize mechanisms and thresholds for nonlinear behavior.

SA159. Effects of Soft Tissue Thickness on Mastoid Impedance and Skull Bone Velocity Evoked by Bone Conduction at 0.1–25 kHz

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Background: Bone conduction (BC) testing is a standard audiometric procedure used to estimate cochlear sensitivity by presenting vibration to the head. The skin, muscle, and soft tissue (SMST) between the BC transducer and the mastoid can alter the force transmitted to bone and thereby influence BC hearing sensitivity. Previous work suggests a frequency-dependent elevation of BC thresholds and attenuation of petrous bone vibration with increasing SMST thickness from 4 to 8 kHz. However, effects at extended high frequencies (EHF; 8–20 kHz) remain unclear. We investigated how SMST thickness impacts the force delivered to the temporal bone by measuring mastoid mechanical impedance and BC-evoked temporal bone velocity.

Methods: We used fresh-frozen cadaveric heads without a history of middle-ear disease. For impedance measurements, heads were placed on a scissor jack and a digital scale on a vibration-isolated table with a horizontal mastoid surface. An impedance head (Brüel and Kjær (BK) 8001) coupled to a mini shaker (BK 4810) was contacted on the mastoid with 5 N static force. Mastoid impedance was calculated from force and acceleration recordings during Chirp stimulation by BK 8001 (0.1–25 kHz), with subtraction of the self-impedance. To

approximate clinically observed SMST thickness (2–20 mm), we modified local SMST by serially adding porcine skin layers (2 mm per layer; 1, 2, 4, 6, or 8 layers).

We also measured the BC-induced velocity of the petrous bone and the bony tympanic membrane annulus (TMA). A BC transducer (KLH96) with a headband providing 5 N static force was positioned over the mastoid at the same site as the impedance testing. After elevating the tympanic membrane, a laser Doppler vibrometer (Polytec) was focused transcanally on the promontory and posterosuperior TMA. Petrous and TMA velocities evoked by BC stimulation (Chirp and tones, 1–20 kHz) were measured for each SMST condition and also via direct bone contact following native SMST removal.

Results: With increasing SMST thickness, mastoid impedance at ≤ 3 kHz decreased, consistent with reduced stiffness. In contrast, impedance changes were small at EHF, despite the added mass. TMA velocity was comparable to promontory velocity across frequencies, supporting the TMA as a practical in vivo proxy site for assessing cochlear vibration with BC stimulation. Added skin layers significantly reduced cochlear (promontory) velocity by as much as ~ 20 dB at ≥ 10 kHz. Direct bone contact boosted cochlear vibration across the entire frequency range tested.

Conclusions: Increased SMST significantly reduced mastoid impedance at lower frequencies and depressed cochlear bone velocity driven by a BC transducer at EHF. These cadaveric data motivate human-subject studies that relate mastoid SMST to EHF BC thresholds using transcanal TMA velocity as a proxy for cochlear vibration in vivo.

SA160. Nonlinear Growth of Middle Ear Transfer Function After Ossicular Chain Reconstruction Using Partial Ossicular Replacement Prosthesis and Stapes Piston

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Background: At moderate sound pressure levels (SPL), the ossicular chain (OC) operates as a linear system to transmit sound energy to the cochlea. At high levels, this transmission becomes nonlinear through active muscle reflexes and passive nonlinear mechanics, which mainly leads to compressive growth of stapes velocity, protecting the cochlea from high-intensity sound. Previous studies of human temporal bones with intact OCs demonstrated the onset of passive nonlinear growth of the middle ear transfer function (METF) at frequency-dependent sound pressure thresholds. In clinical settings, damaged OCs can be reconstructed by ossiculoplasty using prostheses to reestablish ossicular continuity. However, the growth of the METF after ossicular chain reconstruction (OCR) has not been well studied, and it remains unclear whether the nonlinear growth of METF persists in reconstructed OCs over a broad frequency range.

Methods: Fresh-frozen human temporal bones without middle-ear disease were prepared in the standard procedure by opening the facial recess and resecting the stapedial tendon for laser Doppler vibrometer (LDV) measurements on the stapes posterior crus. A probe microphone was inserted through a small hole opened in the anterior canal wall to record the ear canal sound pressure (PEC). A second LDV was focused on the umbo through that hole sealed with a clear plastic window. A short silicone tube connecting to a loudspeaker was then sealed to the ear canal opening to deliver broadband stimuli (tones, 0.2–20 kHz). Two types of prostheses were used for OCR. In full-titanium Partial Ossicular Replacement

Prosthesis (PORP) reconstruction, the incus was removed, the prosthesis head plate was coupled to the tympanic membrane with cartilage grafts, and the clip was coupled to the stapes head. In fluoroplastic stapes piston reconstruction, the entire stapes was removed, the piston was rested on perichondrium covering the oval window, and the wire was crimped to the distal incus long process. The velocity of the umbo and stapes (or piston) was simultaneously measured by LDVs and normalized by PEC to derive their METFs at different PEC levels.

Results: PEC increased linearly in general with driving voltages, reaching as high as 140 dB SPL at > 1 kHz. However, nonlinear PEC growth was observed at the highest stimulus voltage, which was excluded for further analysis of nonlinear growth of METF. Umbo and stapes/piston velocity METFs in both PORP and piston reconstructions showed small-scale nonlinear growth at some high stimulus levels within limited frequency ranges, which differs from nonlinear growth of the intact OC.

Conclusions: Though altered from normal, passive nonlinear METF persists after OCR. Our data allow us to separate the nonlinear effects of the annular ligament (removed by stapes reconstruction) and the incudomalleolar joint (removed by PORP reconstruction).

SA161. The Sensitivity of Nonlinear Ogden Parameters in the Middle-Ear Response to High-Intensity Sounds

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Background: For high-level sounds (i.e., > 130 dB SPL), the middle-ear output is nonlinear, and yet the source of such nonlinearities has not been characterized. Recent finite-element (FE) models capture this nonlinear behavior by assigning hyperelastic material properties either to all middle-ear soft tissues or selectively just the tympanic membrane (TM) (Tubelli et al., ARO2025; Mohammadi et al., 2024). These studies have yet to explore the effect of variations in nonlinear mechanical parameters on the FE model output. This study explores how nonlinear parameters in an FE model affect output, which can help us begin to understand variability across ears.

Methods: A previously developed nonlinear time-domain FE model of the human middle ear consisting of ossicles, TM, and other soft tissues was used (Tubelli et al., ARO2025). All soft tissues were modeled as first-order Ogden hyperelastic materials with two parameters; i.e., shear modulus μ and exponent α (Lohr et al., 2022), and a sensitivity analysis was carried out on these parameters. The TM and other structures with the highest level of strains were identified as the structures of interest (SOIs) for incorporating variability in nonlinear parameters. The acoustic elements of the ear canal were excluded for simplicity, and surface pressure excitations at three pure tone frequencies (500, 4000, and 8000 Hz) and three different levels (120, 150, and 170 dB SPL) were applied directly to the TM surface. For this study, variations of μ and α were investigated on a simplified model and extended to the middle-ear model.

Results: The highest level of strain was observed on the stapedial annular ligament (SAL), which was chosen as one of the SOIs along with the TM. A preliminary sensitivity analysis on the simplified model ($0.01 \text{ MPa} \leq \mu \leq 1.0 \text{ MPa}$ and $5 \leq \alpha \leq 30$) suggests that

displacements generally decreased with increasing both shear modulus μ and exponent α , but μ had a more pronounced effect than α . At the low frequency, displacements decreased with α almost uniformly across all μ values, whereas at higher frequencies, they became more sensitive to α at larger μ values ($\mu \geq 0.1$ MPa). Furthermore, displacements were generally more sensitive to changes in α within its lower range ($\alpha \leq 15$), and more sensitive to changes in μ within its higher range ($\mu \geq 0.1$ MPa).

Conclusions: We have homed in on the first-order Ogden model to investigate the effects of parameters in middle-ear nonlinearity. Based on the results from the simplified model, we concluded that stiffness sensitivity to Ogden parameters is frequency-dependent, which guides us to investigate parameter variations of the TM and SAL on the nonlinear responses of the middle ear.

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SA162. Autonomic Responses to Transcutaneous Auricular Vagus Nerve Stimulation (taVNS): A Pilot Study

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Background: Invasive cervical vagus nerve stimulation is an established therapy for epilepsy and depression but carries risks and costs associated with implanted medical devices. Transcutaneous auricular vagus nerve stimulation (taVNS) offers a non-invasive alternative by targeting the auricular branch of the vagus nerve. While promising, the mechanism mediating taVNS's effects remain poorly defined. This pilot study seeks to explore the physiological effects of taVNS across both common autonomic measures including heart rate (HR), mean arterial pressure (MAP), heart rate variability (HRV) with other promising autonomic measures such as photoplethysmography (PPG), and galvanic skin response (GSR).

Methods: After obtaining informed consent (IRB#20190769), six healthy participants underwent taVNS (Spark Biomedical, Spark Sparrow Ascent) at the left cymba (20 Hz, 500 μ s pulse width) titrated to their perceptual threshold. The protocol consisted of a 4-minute baseline recording, 16-minutes of stimulation (eight 1min on/off duty cycles), and a 10-minute post-stimulation observation period. Continuous data for ECG, bilateral finger PPG, and bilateral palm GSR was collected to analyze HRV, pulsatile component of the PPG, and eccrine sweat response from GSR respectively. Tukey-Kramer post-hoc analysis of repeated-measures ANOVA (rANOVA) was used for HR and MAP for each epoch (baseline vs. stimulation vs. observation). PPG and GSR data were z-scored with respect to a 10 second pre-stimulation baseline for each duty cycle to account for baseline drift in signal. These standardized traces were averaged across subjects and cycles for further assessment. Quantitative analysis of GSR slope, PPG area-under-the-curve, and HRV are ongoing to investigate the preliminary qualitative assessment that taVNS decreased skin conductance and peripheral blood flow correlates.

Results: Post-hoc Tukey-Kramer analysis found a statistically significant decrease in HR between all epochs suggestive of a lingering effect of taVNS. Specifically, baseline vs. stimulation comparison revealed a decrease of 1.0 beat per minute ($p = 0.044$) and

stimulation vs. observation epochs revealed a decrease of 1.23 beats per minute ($p = 0.04$). rANOVA analysis found no significant effect for MAP ($p=0.809$). Standardized PPG and GSR traces demonstrated clear, time-locked responses to stimulus onset and termination. GSR exhibited a characteristic decrease during stimulation followed by a sharp increase upon cessation. PPG waveforms showed a decrease in amplitude during stimulation which could correlate with transient vasoconstriction with a subsequent rebound in signal upon offset.

Conclusions: taVNS at the left cymba for eight 1min on/off duty cycles (20 Hz, 500 μ s pulse width) elicits a potential decrease in heart rate without altering blood pressure, suggesting a specific modulation of cardiac control. Concurrently, observed GSR and PPG responses indicate a complex interplay with sympathetic pathways. These preliminary results suggest taVNS under this paradigm triggers a mixed autonomic response profile. Further quantitative analysis will better characterize the sympathetic contribution to these effects.

SA163. Mapping the Ossicular Chain Vibrations With 3D Optical Coherence Tomography

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Background: The vibrations of the ossicular chain in the middle ear in response to acoustic stimuli are not yet fully understood because of several technological constraints. Conventional methods to study these vibrations usually involve Laser-Doppler Vibrometry (LDV), and even though three-dimensional (3D) LDV set-ups exist, this technique remains restricted to vibrational measurements of only the dominant reflector along the beam path. Optical-Coherence Tomography Vibrometry (OCTv), in contrast, allows for volumetric imaging and spatially resolved vibrational measurements at a depth below the tissue surface. Hence, the vibrational information is available at the voxel-level, surface and sub-surface.

Methods: To evaluate the complex 3D vibratory patterns of the ossicular chain, we developed a framework for 3D OCTv measurements in a human temporal bone using a single OCT beam mounted on a 6 degrees of freedom robot arm that allows rotating the OCT beam around a fixed specimen. By combining vibrometric measurements from multiple angles using volume registration methods and vector calculus, all cartesian components of the 3D vibrations of the ossicular chain can be expressed in an anatomically relevant orthogonal coordinate system. As some regions of the ossicular chain remain optically inaccessible despite a maximally exposing surgical preparation, the integral evaluation of the ossicular chain vibrations requires some additional processing steps.

Results: Here we present data of multiple 3D OCTv measurements in one fresh-frozen human temporal bone specimen. Optically inaccessible regions were attributed a value through an interpolation method and finite-element modeling. Sub-surface vibrometric data was corrected for refraction. Ossicular oscillations were induced through an ER3+ insert phone in the external ear canal at stimulus frequencies of 0.5, 1, 3, 6 and 10 kHz and ear-canal pressures were measured with a ER7 probe-mic for reference. The presented data has a depth resolution of 4 μ m and a lateral resolution of 8 μ m. The cartesian components of the 3D vibrations are accurate to approximately -20dB re 1mm/s.

Conclusions: Our 3D OCTv set-up allows untangling the complex vibratory patterns of the ossicular chain. It will pave the way for an integral understanding of how the middle ear transfers acoustic energy in response to different sound frequencies. These insights will prove

valuable for the optimization of middle ear microphones and other future hearing technologies that benefit from or augment middle ear functionality.

SA164. Novel Material-Optimized, Ct-Guided 3D Printing of Tympanic Membrane Prostheses

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Background: Tympanoplasty—reconstruction of the tympanic membrane (TM) and ossicular chain—is well established, yet hearing restoration remains inconsistent. Patient-specific, 3D-printed prostheses may offer superior anatomical fit and functional outcomes.

Methods: A cadaveric temporal bone was imaged using computed tomography (CT). Based on these scans, a custom TM–ossicle prosthesis was designed and fabricated with 3D printing. Experimental eardrum patches were produced using lab-developed ECC FLEX 00-30 material in varying ratios. TM motion in response to 60–90 dB SPL sound stimuli was assessed with laser Doppler vibrometer (LDV), and mechanical properties of the printed TMs were evaluated with standardized mechanical testing.

Results: At 600 and 1000 Hz under 60–90 dB SPL input, the 3D-printed TM demonstrated superior mechanical performance compared to conventional TM patch materials. The mechanical performance is related to the materials and the approach to connection of the TM with ear canal wall.

Conclusions: CT-based, 3D-printed TM–ossicular prostheses incorporating novel materials can be successfully designed and fabricated. These results establish feasibility and underscore the potential of patient-specific, material-optimized 3D-printed TMs as a future treatment for conductive hearing loss caused by TM perforations or ruptures.

SA165. DX243 Weakens Acoustic Trauma-Induced Cortical Brain Oscillation Changes and Distinct Neurocochlear Impairments

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Background: A dramatic shift in societal demographics will lead to rapid growth in the number of older people with hearing deficits. The increasing risk of cognitive decline predicted to be associated with hearing loss has become a key challenge for future societies. Over age accumulating acoustic injury contributes particularly to speech discrimination deficits in background noise, a feature predicted to be linked to the high vulnerability of auditory fibers with low spontaneous discharge rate and high activation thresholds.

Methods: We here investigate a new potential drug, dendrogenin, which is predicted to exhibit otoprotective function and balanced neurite outgrowth. We test the effect of dendrogenin on cortical local field potentials (LFP) and peripheral paradigms after acoustic trauma (AT) in the rat animal model.

Results: In line with previous studies, AT reduced acoustic stimuli-induced local field potentials in the auditory cortex 2 weeks after AT, while neither acoustic stimuli nor AT effected LFP in the prefrontal cortex, visual cortex and hippocampus. 2 weeks after daily injection of DX but also 6 weeks after the last DX injection a dose dependent protective effect of DX could be observed on AT induced decline of LFP spectral power. DX did not affect hearing thresholds after AT but exhibited with the same dose response as shown for its effect on LFP, a protective effect on auditory fiber functions. A significant preservation of IHCs ribbon numbers after AT, less impairment of specifically noise-sensitive auditory fiber processing (tested through Notch-noise) and a positive preserving effect on pre-postsynaptic integrity during multi-click responses with decreasing time intervals, suggest a possible selective effect of DX on the most vulnerable low-SR auditory fiber type.

Conclusions: These findings suggest dendrogenin as a potential neuroprotective drug for the treatment and prevention of age-and noise-induced hearing loss.

SA166. Mitochondrial Transplantation as a Strategy to Reduce Noise-Induced Hearing Loss

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Background: Hearing loss is a prevalent and disabling condition with no curative treatment, highlighting the urgent need for new therapeutic strategies. Mitochondrial transplantation, the delivery of exogenous mitochondria into cells, has shown promise in mitigating mitochondria-related disorders. However, despite the central role of mitochondria in auditory function, this approach has not yet been explored for hearing loss.

Methods: Exogenous mitochondria were isolated from auditory cells and transferred into a new set of auditory cells following oxidative stress to evaluate their biological consequences. In vivo, isolated exogenous mitochondria were injected into the cochlea of mice, and their localization and impact on hearing after noise exposure were assessed.

Results: Exogenous mitochondria from HEI-OC1 auditory cells were internalized in a dose-dependent manner without inducing toxicity. They enhanced bioenergetics, protected against oxidative stress, and reduced H₂O₂-induced apoptosis. In vivo, injected mitochondria localized to cochlear sensory cells, were well tolerated, and conferred protection against noise-induced hearing loss.

Conclusions: These findings provide the first evidence supporting the feasibility and therapeutic potential of mitochondrial transplantation in auditory cells. If validated in animal models and ultimately in humans, this strategy holds strong promise as a novel treatment for sensorineural hearing loss.

SA167. Dual Photothermal and Magnetically Assisted Transport of Magnetic Nanoparticle Drug Delivery Vehicles

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Background: Magnetic nanoparticles (MNPs) can enhance round window membrane (RWM) penetration via magnetically assisted transport for cochlear drug delivery. However, custom synthesized MNPs also have photothermal properties that can play a dual role in

augmenting RWM transport by enhancing diffusivity. We investigate the interaction between particle size, temperature, and magnetic field strength in MNP transport across an acellular, semipermeable membrane.

Methods: Superparamagnetic Fe₃O₄ MNPs were synthesized with core diameters of 6-10nm and 10-15 nm to achieve differential magnetic susceptibility, followed by grafting with PEG3000 to achieve hydrodynamic sizes of ~100 nm and ~140 nm. MNP characterization was performed using transmission electron microscopy, and zeta sizer. Decellularized porcine intestinal submucosal (SIS) membranes were mounted in a dual-chamber diffusion device. The microfluidic device, analogous to a Ussing chamber, was developed specifically to allow both application of a magnetic field gradient and laser-induced photothermal activation of MNPs at the membrane. MNP suspensions in phosphate buffered saline were tested under magnetic field application using a neodymium bar magnet and/or laser irradiation (606–1342 nm) tuned to hold interface temperature ranging between 31–45 °C. MNP concentration across the SIS membrane was measured by assaying iron content as a function of time, magnetic field gradient, temperature, and MNP size, with a view towards extending these investigations into an ex vivo guinea pig round window membrane model.

Results: Completed SIS studies yielded measurable transport across MNP size ranges, with the 100 nm MNPs achieving higher cumulative transport and larger permeability compared to 140nm MNPs. Laser-activated photothermal stimulation elevated the temperature at the SIS membrane interface from ~31 °C to ~42–45 °C, with increased MNP transport versus isothermal controls. Superimposing a magnetic field further enhanced delivery (21%) with transport decreasing as magnet–membrane distance increased, consistent with reduced field gradients; across conditions, photothermal and magnetic inputs were additive without evidence of thermal damage at the tested temperatures.

Conclusions: Magnetic and photothermal activation are dual modes of transport augmentation based on chemical physical properties of MNPs that can enhance RWM penetration. We establish a benchtop system to investigate photothermal activation of MNPs in conjunction with permeability measurements. Findings here demonstrate parameters important in designing MNP systems capable of magnetic and photothermal assisted transport, and in an acellular semipermeable membrane, the relative contribution of each to cumulative transport. These findings will serve as a basis for investigations in preclinical ex vivo RWM models and MNP vehicles with therapeutic payload.

SA168. Proteome Comparison Between Endolymph and Perilymph in Mice

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Background: The precise regulation of inner ear fluids is essential for auditory function, yet the molecular mechanisms that maintain endolymph/perilymph homeostasis remain poorly understood. Endolymphatic hydrops, an abnormal expansion of the scala media, is a landmark of Ménière's disease. Despite the importance of protein to maintain osmotic pressure, the protein composition of endolymph relative to perilymph has not been systematically compared in healthy and disease states. Characterizing these differences is a critical first step toward understanding how dysregulation of fluid compartments contributes to disease pathology. This study defines and compares the baseline protein content of endolymph and perilymph in wild-type mice as a foundation for future comparisons in a hydrops mouse model.

Methods: Endolymph and perilymph samples were collected from the cochleae of 6 adult wild-type mice using a micropipette. Approximately 1 microgram of protein was reduced and alkylated, followed by trypsin digestion. Each sample was then run on a Thermo Q Exactive mass spectrometer coupled to a Waters M-Class nano-liquid chromatography system. The raw files were searched with Proteome Discoverer. GO term analysis was performed in PANTHER.

Results: 569 proteins out of 735 proteins identified were common to both endolymph and perilymph. Our findings suggest that endolymph and perilymph maintain separate protein environments. Our data highlight differences that may be perturbed in pathological states such as endolymphatic hydrops

Conclusions: Our study provides a direct comparison of protein content between endolymph and perilymph, establishing a baseline for future studies in mutant models of endolymphatic hydrops. Protein homeostasis between endolymph and perilymph is likely disrupted during hydrops formation. Understanding these differences will inform hypotheses about how fluid imbalance leads to altered cochlear function and hearing loss. Future research will extend this work to hydrops models to identify disease-specific proteomic shifts.

SA169. Low-Intensity Ultrasound Stimulation Enhances Fluid-Regulating Channels in the Inner Ear

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Background: Maintenance of endolymphatic homeostasis is critical for cochlear function and relies on the coordinated regulation of water and ion channels. Aquaporins (AQPs) have been implicated in inner ear fluid balance and in the pathophysiology of disorders such as Meniere's disease. Low-intensity ultrasound (LIUS) has emerged as a non-invasive tool capable of modulating mechanosensitive pathways in various tissues. Our previous work demonstrated that LIUS augmented glymphatic influx through the TRPV4-AQP4 signaling pathway in astrocytes, thereby promoting cerebrospinal fluid influx into the perivascular spaces and enhancing interstitial substance clearance from brain parenchyma without inducing tissue damage. Based on these findings, we hypothesized that LIUS may similarly regulate fluid homeostasis in the cochlea through modulation of aquaporins and mechanosensitive transient receptor potential (TRP) channels.

Methods: Cochlear explants were prepared from postnatal day 6 C57BL/6 mice and exposed to LIUS stimulation. Cochlear explants were fixed at prespecified post-stimulation time points (0, 30, 60, and 120 mins) after stimulation and processed for immunofluorescence. Expression and subcellular localization of AQP4, AQP5, and TRPV4 were evaluated in different regions of cochlear explants (mainly around the organ of Corti) using confocal microscopy. Signal intensity and distribution were compared with non-stimulated controls.

Results: LIUS stimulation induced distinct changes in the expression and localization of water and mechanosensitive channels. AQP4 expression increased in Hensen's cells, Deiters' cells, and Kölliker's organ compared with controls at 30 minutes after stimulation. AQP5 exhibited rapid redistribution from the cytoplasm to the apical membrane of supporting cells, with signal enhancement observed within 30 minutes after stimulation. In addition, AQP5

translocation to the cytoplasm and basal regions was observed in LIUS-treated explants. On the other hand, TRPV4 expression also increased following LIUS immediately (at 0 min) after stimulation, particularly in Kölliker's organ. Together, these findings suggest that LIUS activates mechanosensitive signaling pathways leading to selective trafficking and upregulation of AQP4, AQP5 and TRPV4 in cochlear explants.

Conclusions: Our data demonstrate that LIUS modulates AQP4, AQP5 and TRPV4 expression in cochlear explants, implicating these proteins in ultrasound-mediated regulation of fluid homeostasis. These results provide mechanistic insight into LIUS-mediated control of inner-ear fluid dynamics and support further study of LIUS as a non-invasive strategy to correct endolymphatic imbalance in hydrops-related disorders.

SA170. Utility of Cochlear Perilymph Biomarkers for Hearing Loss - A Systematic Review

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Background: The live cochlea has long been considered a relatively inaccessible and 'sacred' organ with traditional sampling techniques posing a risk to hearing and balance functions. Researchers' ability to investigate the pathogenesis and treatment of sensorineural hearing loss has subsequently been limited, commonly involving in vitro models, translational animal models, and post-mortem analysis instead. Previous human studies investigating sensorineural hearing loss have also been restricted to using peripheral blood, urine, or cerebrospinal fluid samples to indirectly measure inner ear biomarkers. More recently, the establishment of a novel and safe technique for intraoperative perilymph sampling has enabled the collection of a 'liquid biopsy' of the cochlea while still preserving inner ear function. This crucial development has opened avenues for more accurately characterising the complex cochlear microenvironment and investigating the mechanisms of sensorineural hearing loss more directly. Perilymph sampling also has the potential to provide insight into why postoperative outcomes may vary between cochlear implant users who have otherwise similar preoperative clinical characteristics. The present systematic review therefore aimed to elucidate the gap in the literature regarding the utility of perilymph biomarkers in sensorineural hearing loss diagnosis, treatment, and prognosis.

Methods: A search was conducted using the Medline, Embase, Web of Science, and Scopus databases from inception to April 2024, in keeping with PRISMA-S guidelines. Keywords were divided into two groups according to biomarker type (biomarker; metabolome; proteome; RNA; miRNA; inflammasome) and sensorineural hearing loss pathologies (hearing loss; deafness; Ménière; otosclerosis). Of the 7471 studies initially identified, 15 met the inclusion criteria. A ROBINS-I tool was used to evaluate the risk of bias in each of the included studies.

Results: All 15 studies included were correlational in design, with the majority of studies sampling perilymph in sensorineural hearing loss patients via the round window during cochlear implantation. Due to the functional diversity of perilymph biomarkers identified in this review, biomarkers were grouped into six main categories according to their underlying pathological processes: heat shock proteins (HSPs), immune/inflammatory mediators, microRNAs, neurotrophic proteins, metabolomes, and structural proteins. For each category, key potential biomarkers were identified and analysed for consideration in future studies

utilising perilymph sampling. In particular, HSP-70, HSP-90, complement components, miR-1299, miR-1270, and brain-derived neurotrophic factor regulated proteins may help to direct future studies focused on characterising the biomarker profile in patients with sensorineural hearing loss.

Conclusions: Future targeted studies with larger cohorts and clearer hearing loss aetiologies are required to help further characterise the prevalence and functional role of these perilymph biomarkers. Such studies may help to pave the way towards a more accurate understanding and diagnosis of sensorineural hearing loss pathologies, improved prediction of cochlear implantation prognosis, and targeted therapeutic management.

SA171. Immune Response in the Spiral Ganglion Following Kanamycin-Induced Hair Cell Loss

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Background: Spiral ganglion neurons (SGNs) of the cochlea gradually die after neonatal kanamycin-induced hair cell loss in rats, resulting in the death of >80% of all SGNs over the course of ~14 weeks, apparent by postnatal day 39 (P39). We have previously shown that macrophages increase in abundance and activation by P21, well prior to the start of significant SGN death. Concurrent with the apparent onset of SGN death, there is an increase in lymphocytes in the ganglion, including CD4+ helper T cells, CD8+ cytotoxic T cells, and CD161+ NK cells. To determine whether the lymphocyte component of this inflammatory response is causal to SGN death, we evaluated SGN survival after hair cell loss in two immunodeficient rat strains: 1) RNU nude rats lacking T cells and 2) SRG rats lacking T, B, and NK cells.

Methods: Sprague-Dawley (SD), Crl:NIH-Foxn1^{rnu/rnu} (RNU nude, T cell deficient), Crl:NIH-Foxn1^{rnu/+} (heterozygotes, normal T cell population), and Rag2^{em2}hera^{fl2}rgem1^{hera}/HblCrl (SRG, T, B, NK cell-deficient) rats were intraperitoneally injected 1x/day with kanamycin from P8 to P16. Cohorts of deafened and hearing control rats were euthanized at various ages and cochlea were sectioned for immunohistochemistry to label hair cells, neurons, leukocytes, macrophages, T cells, B cells, and NK cells.

Results: We found that SGN survival in RNU nude rats is not significantly different than in RNU+ rats, indicating that T cells are not required for SGN death after hair cell loss. Genetic ablation of T, B, and NK cells in SRG rats does not prevent SGN death after hair cell loss but does moderately improve survival in the basal half of the cochlea relative to kanamycin-treated wildtype conspecific controls. For all strains evaluated, we also found that the mutations do not affect the typical increase in macrophage number in kanamycin treated groups.

Conclusions: We directly assessed the role of T cells in SGN death after deafening using T cell deficient RNU nude rats and found that T cells are not required for SGN death after hair cell loss. Using SRG rats, we found that lack of all lymphocytes improves SGN survival in the basal, but not apical, half of the cochlea, revealing regional differences in the role of immune cells in cochlear neurodegeneration. As we have previously reported, the increase in macrophage number and activation within the cochlea precedes significant neuronal death, implying that macrophages are not simply responding to increased need for phagocytosis of neuronal debris, but rather may be casual to the death of SGNs. Studies using the CSF1R

inhibitor, PLX5622, are currently underway to assess the role of macrophages in SGN death after hair cell loss.

SA172. Hearing Loss–Associated Variant p.V60L (c.178G > T) in P2RX2 Alters Calcium Flux in Human Inner-Ear Organoids

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Background: Mutations in P2RX2, which encodes an ATP-gated ion channel, are responsible for a disease known as autosomal dominant deafness 41 (DFNA41) and contribute to susceptibility to noise-induced hearing loss (NIHL). Herein we examined the impact of the first human P2X2 channel mutation identified, the p.V60L substitution, in human inner-ear organoids.

Methods: The P2RX2 p.V60L variant was introduced by CRISPR/Cas9 into human induced pluripotent stem cells (hiPSCs) to an isogenic control line. Inner-ear organoids were derived from both lines. Hair cell induction was monitored by immunocytochemistry, and calcium flux was assessed using Fluo-4 dye.

Results: Organoid induction was not affected by the P2RX2 variant. Hair cells were present in both wild-type (WT) and P2RX2 p.V60L organoids without evidence of degeneration through day 60 of culture. Calcium imaging assays showed reduced baseline calcium activity, as well as a diminished response to ATP, in organoids carrying the p.V60L variant.

Conclusions: Human inner ear organoids provide a useful platform for studying the functional consequences of hearing loss–associated genetic variants. The P2RX2 p.V60L variant reduces calcium signaling in human inner ear cells, which implicates the role of the P2RX2 gene in the proper function of inner ear.

SA173. Characterizing the Role of P2Y Metabotropic ATP Receptors in Medial Olivocochlear (MOC)-Mediated Effects on DPOAEs

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Background: Originating in the superior olivary complex, medial olivocochlear (MOC) neurons project across the brainstem and out cranial nerve VIII to synapse on outer hair cells (OHCs) along the cochlear spiral. MOC activation produces a characteristic suppression and enhancement of distortion product otoacoustic emissions (DPOAEs), presumably through actions on the cochlear amplifier. During MOC stimulation, MOC-mediated suppression (MMS) occurs within seconds, whereas the onset and offset kinetics of MOC-mediated enhancement (MME) are distinctly more protracted. MMS requires the release of ACh from MOC terminals and activation of postsynaptic $\alpha 9\alpha 10$ nicotinic ACh receptors ($\alpha 9\alpha 10$ nAChRs) on OHCs. While response kinetics suggest metabotropic receptor signaling, the mechanisms underlying MME have not been identified. Beyond ACh, existing data have revealed that MOC neurons may express nearly a dozen other transmitters including ATP, catecholamines, GABA, glycine, serotonin, CGRP, opioids, and neuropeptides. Previous studies, however, have revealed that MME does not appear to rely on adrenergic, CGRP, dopaminergic, GABAergic, glycinergic, muscarinic ACh, or opioid signaling. Motivated by the metabotropic-like kinetics of MME and evidence that MOC efferents co-release ATP, we

have focused our efforts on investigating if ATP and its activation of P2Y metabotropic receptors (P2YRs) play any role in the generation of MME.

Methods: To evaluate the role of ATP and P2YRs on MOC-mediated effects, we recorded DPOAEs in urethane/xylazine-anesthetized C57Bl/6 mice while stimulating MOC neurons in the 4th ventricle before, during, and after the administration of selective P2YR agonists and antagonists. DPOAE stimulus generation and measurements were made using Tucker-Davis System hardware and custom software, using F1 and F2 frequencies (10 and 12 kHz, respectively) delivered to the ear through Etymotic ER2 earphones and recorded via an ER-10B+ microphone. To isolate MME, we eliminated MMS using selective $\alpha 9\alpha 10$ nAChRs antagonists or by performing parallel experiments in $\alpha 9$ KO mice. Most P2YR compounds were delivered directly into the perilymph using an intracanal (IC) cannula inserted through the bony posterior semicircular canal.

Results: Previous studies have indicated that the P2YR subtypes P2YR1 and P2YR4 are found on OHCs while P2Y2Rs are restricted to several classes of supporting cells. Guided by this, we used subtype-selective agents to probe each receptor subfamily. P2YR1 antagonists enhanced MMS while P2YR1 agonists attenuated MMS suggesting there may be some interactions between P2YR1s and $\alpha 9\alpha 10$ nAChRs on OHCs. The IC administration of P2YR2 and P2YR4 agonists mimicked MME and, in some instances, appear to reduce it at later time points, in what might be evidence for desensitization. P2YR2 and P2YR4 compounds appear to also have varying effects on MMS.

Conclusions: This study attempts to characterize the role of P2YRs in MME and preliminary data suggesting that P2YR2s and possibly P2YR4s are involved. Further pharmacological assessment and complementary genetic approaches are warranted to define receptor-specific mechanisms and kinetics.

SA174. Cholinergic Modulation of Membrane Properties of Type II Vestibular Hair Cells

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Background: In the mammalian peripheral vestibular system, cholinergic efferent fibers from the brainstem innervate type II hair cells (HC-II) and afferent terminals in the inner ear. Previous studies have established that HC-II express $\alpha 9$ -containing nicotinic acetylcholine receptors (nAChRs), which trigger rapid hyperpolarization through activation of small conductance calcium-activated potassium (SK) channels (Poppi et al., 2017; Yu et al., 2020). In contrast, very little is known about muscarinic receptor (mAChR) signaling in HC-II, particularly in mammals. The only available data come from studies on enzymatically dissociated guinea pig HC-II, where the investigators themselves note that the dissociation process significantly alters the cells' physiological properties (Kong et al., 2005; 2007). Here, we examined whether HC-II cells respond to mAChR activation and characterized the nature of their response.

Methods: We used a whole tissue preparation and performed whole-cell patch-clamp recordings from HC-II in the cristae of the horizontal and anterior semicircular canals of wild-type C57BL/6 mice or mice lacking large conductance potassium (BK) channels at P13–P17. Pharmacological agents included IBTX (150 nM), oxotremorine methiodide (Oxo-M, 20 μ M), and apamin (300 nM), all bath-applied during experiments. Putative HC-II were identified by the absence of surrounding calyces under differential interference contrast

(DIC) optics and subsequently confirmed by their characteristic current profiles in response to depolarizing steps.

Results: HC-II displayed voltage-dependent potassium currents when subjected to voltage steps from -124 mV to $+16$ mV in 20 mV increments. These responses included I_h currents at -124 mV, small currents near the resting potential of -59.2 ± 3.5 mV ($n = 12$), and large outward currents (up to 3 nA) during depolarizing steps. Application of Oxo-M significantly reduced these outward currents ($n = 9$) at the most depolarized steps (two-way repeated measures ANOVA, $p < 0.0001$; Bonferroni post hoc: $p < 0.0001$ for steps > -20 mV). Occlusion experiments with IBTX followed by Oxo-M plus IBTX demonstrated that IBTX alone reduced outward currents at steps > 16 mV, and co-application of Oxo-M did not cause further reduction ($n = 8$). Furthermore, apamin failed to block Oxo-M effects ($n = 5$). Confirming these findings, HC-II of BK KO mice (Meredith et al., 2004) showed smaller outward currents at depolarized membrane potentials compared to WT mice and were not affected by Oxo-M.

Conclusions: Our results show that mAChR activation inhibits BK channels at depolarized potentials, increasing HC-II excitability at > 0 mV during large cilia deflections. In contrast, activation of $\alpha 9$ -containing nAChRs engages SK channels to suppress excitability below 0 mV. Therefore, simultaneous activation of mAChRs and nAChRs by efferent input may act as a high-pass filter, enhancing responses to strong stimuli while dampening weaker ones.

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SA175. Do Hensen's Cells in the Organ of Corti Signal Tissue Injury Through Type II Spiral Ganglion Neurons?

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Background: The cochlea, a spiral-shaped structure in the inner ear, contains the organ of Corti, which converts mechanical vibrations into electrical signals through specialized sensory hair cells. Spiral ganglion neurons (SGNs) innervate hair cells to relay information to the brain and are categorized into type I or type II neurons. Type I neurons are myelinated, innervate inner hair cells (the 'true' auditory hair cells), and represent most of the spiral ganglion neurons ($\geq 90\%$). Type II neurons are unmyelinated, innervate multiple outer hair cells simultaneously (the cells responsible for sound amplification within the cochlea), and their function is not well understood but they appear to respond to potentially damaging stimuli (nociception) like loud noise. The transient receptor potential cation channel A1 (TRPA1) is a well-established sensor of tissue injury found in pain-sensing neurons throughout the body. TRPA1 is also expressed in the cochlea, exclusively in Hensen's cells. Notably, type II SGNs have been shown to make electron-dense contacts with Hensen's cells in guinea pigs, but whether these contacts represent functional synapses and/or are present in other mammals is unknown. Here, we are exploring the potential innervation of Hensen's cells by type II SGNs in mice.

Methods: We are using immunolabeling against neurofilament heavy chain (NF-H) and confocal microscopy of the adult (> 4 weeks old) organ of Corti from wild-type mice (C57Bl/6). To explore the innervation patterns of SGNs along the entire cochlear length (with focus on the outer hair cell region and the neighboring supporting cells) we are using fiber tracing features in Imaris software. We also use whole cochlear mounts from 2- to 3-week old

mice that express the calcium indicator GCaMP6s in neurons to assess calcium activity in SGNs upon the delivery of TRPA1 agonists.

Results: Immunolabeling and confocal imaging revealed the presence of presumed type II SGNs within the Hensen's cell region in the mouse cochlea, and we are currently exploring the anatomical presence of potential synapses. Our preliminary results with the application of the potent TRPA1 agonist para-benzoquinone to the apical turn of the organ of Corti show long-lasting calcium responses in the soma of a small subset (~10%) of SGNs. We are currently evaluating morphological features of these neurons to see whether they indicate a type I or II phenotype.

Conclusions: Our data indicate that Hensen's cells detect injury in the cochlear epithelium and might transmit nociceptive signals to the central nervous system via type II SGNs, making Hensen's cells a novel component of the elusive cochlear nociceptive pathway. Current efforts are focused on determining the cellular and molecular mechanisms of the potential interaction between Hensen's cells and type II SGNs.

SA176. Surface “Painting” of Extracellular Vesicles to Enhance Inner-Ear Tropism

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Background: Extracellular vesicles (EVs) are promising carriers for regenerative therapy in the inner ear, but their native biodistribution favours clearance organs (liver/spleen) and yields limited, non-selective cochlear uptake after intratympanic delivery. We propose post-production surface functionalisation (“painting”) of EVs to increase tropism for key cochlear targets while preserving vesicle integrity and safety. To engineer EV surfaces with carboxylic linker targeting ligands that enhance binding and uptake by the organ of Corti, spiral ganglion neurons and supporting cells, thereby improving delivery and therapeutic efficiency.

Methods: Machine learning-adapted peptide design was used to increase properties as desired to enhance EV trafficking efficiency. To train prototype deep learning model, we used a range of 12 amino acid peptides that bind to hair cells and components of the organ of Corti as identified via phage panning experiments. EVs produced under controlled conditions were “painted” by introducing carboxylic conjugation to anchor the tissue targeting peptides on the surface of EVs. Characterisation included nanoparticle tracking analysis and assessment of efficacy in in vitro and in vivo models.

Results: When compared to unmodified EVs, an increased up-take in HEI-OC was observed. In vivo, selective localisation to hair cells and spiral ganglion neurons or glial cells was achieved with a markedly reduced off-target accumulation.

Conclusions: Current investigations are now focused on critical quality attributes such as ligand identity, density, orientation, vesicle size/charge and binding potency in addition to preservation of hearing thresholds and local tolerability. Although still in its infancy, surface-painted EVs provide a modular, GMP-compatible route to precision delivery in the inner ear, enabling lower doses, fewer off-target effects and clearer mechanism-of-action readouts.

SA177. Bridging the Translational Valley of Death in Auditory Gene and Drug Therapies

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Background: Gene therapies targeting OTOF mutations represent the most advanced and clinically validated approach, showing functional recovery in children. Despite this and exciting advances in auditory science, ototoxicity prevention, and regenerative approaches, approved pharmacological therapies for hearing loss remain scarce due to high attrition rates. The only FDA-approved drug, sodium thiosulfate (PEDMARK®), is limited to preventing cisplatin-induced ototoxicity in pediatric patients. For most individuals, hearing aids and cochlear implants remain the standard of care. High attrition rates in therapeutic development programs are seen across disease areas and often referred to as the translational valley of death, attributed to a disconnect between basic research and the development of safe, effective therapies.

Methods: We drew on our collective experience supporting drug development from discovery through IND-enabling studies to identify recurring barriers and strategies to mitigate translational risk in auditory gene and drug therapies.

Results: Translation failures arise from multiple factors, including animal model limitations, endpoints/biomarkers, scientific culture, novel modalities, and logistics.

Species and model limitations: Animal models differ markedly from humans in cochlear size, hair cell distribution, hearing range, regenerative capacity, and round window permeability. These differences hinder extrapolation of pharmacokinetics and efficacy.

Endpoints and biomarkers: Standard preclinical measures are animal-specific, e.g., ABR and DPOAE need to be aligned to capture subjective human outcomes (e.g., speech discrimination, tinnitus). Post-mortem histopathology often cannot be aligned with clinical symptoms.

Scientific and cultural misalignments: industry focus on translation builds on a prioritization of reproducibility, scalability, and effect size. Conversely, academia's focus on publications prioritizes novelty and statistical significance over robust translational foundations.

Novel modality challenges: Gene and cell therapies introduce risks of off-target effects, immune responses, and vector-related toxicity that are poorly modeled in small animals. Limited species cross-reactivity for biologics and oligonucleotides necessitate surrogate systems with uncertain predictive validity. Regulatory frameworks for these modalities remain underdeveloped.

Operational and logistical hurdles: GMP manufacturing, formulation stability, and inner ear delivery introduce complexities absent at the bench. Clinical trial execution is slowed by patient recruitment, endpoint selection, and statistical design.

Conclusions: The translational gap in auditory therapeutics is especially acute for academic startups, and reflects intertwined scientific, methodological, cultural, and operational challenges. Success requires early and sustained integration of basic scientists with translational experts, including toxicologists, pharmacologists, and regulatory specialists, so that promising discoveries can be de-risked and advanced efficiently toward clinical application. Consistently bridging the translational valley for auditory therapies will require strategic use of large animal models, novel translational biomarkers, and patient-derived

systems to improve human relevance. Emerging in vitro, in silico, and AI-based approaches hold promise, but broad validation and regulatory acceptance are still needed. Early integration of these tools can help de-risk development and accelerate safe, effective therapies to patients.

SA178. Deep Learning Techniques Enable Spatiotemporal Analyses of Cochlear Dynamics

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Background: Due to its ability to visualize structures and characterize motion in the ear, optical coherence tomography (OCT) has emerged as a popular modality to measure cochlear mechanics. Most existing studies characterize the motion of structures within the organ of Corti by plotting tuned responses from single points or multiple selected points. However, OCT offers the ability to measure vibrational data from every point within a volume image stack, and for the most part, these data have remained largely unused due to the massive amount of data available and the time it would take to analyze it. Here, we present a deep learning model that can automatically segment inner ear regions from OCT volumes thus enabling analysis of motion within a complete volume of the cochlea across different anatomic structures.

Methods: We used our 1D-OCT system to image volumes (1mm x 1mm x 1.2mm) of the apical cochlea in 28 mice. In 27 volumes, we manually segmented Reissner's membrane, spiral limbus, tectorial membrane, outer hair cell region, inner hair cell region, Deiters' cell region, tunnel of Corti, lateral compartment, and basilar membrane. The data was split into train, validation, and test sets of 20, 4, and 3 volumes respectively. We trained a 2.5D U-Net model on patches (0.24mm x 0.18mm) sampled from cropped versions of each volume to balance background and foreground regions. After training, the model generated predictions for each patch of a volume using a sliding window approach and retaining the most confident prediction at overlaps. Model predictions were refined using filtering and smoothing to improve consistency across slices. We used the model to segment the 28th volume and analyzed the vibratory patterns of different cochlear structures.

Results: Reflecting its ability to locate structures, the model achieved a foreground dice score of 0.73 and a foreground 95th percentile Hausdorff distance of 26.2 micrometers. We segmented a cochlea dataset with vibrometry measurements across one-third of an apical turn. Within each structure, points at a given radial location were grouped into 3-unit XY bins and averaged to yield a single magnitude and phase, which were then used to create a movie of the vibratory patterns. Movement of the Reissner's membrane and the spiral limbus lacked the appearance of a traveling wave whereas the other structures exhibited an apically traveling wave, with the tectorial membrane showing the greatest vibratory magnitude.

Conclusions: Here, we demonstrate the ability of deep learning methods to segment structures in OCT volumes of the inner ear. In doing so, we leverage deep learning to facilitate spatiotemporal analysis of our vibrometry data for better understanding of cochlear mechanics. The model framework serves as a foundation for future projects leveraging deep learning to analyze biological datasets at scale.

SA179. Deep Learning–Based Classification of Cochlear Pathologies Using ABR Waterfall Plots From Chinchilla Models

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Background: Accurate diagnosis of specific cochlear pathologies remains challenging. Although Auditory Brainstem Response (ABR) testing is widely used to assess hearing function, certain auditory deficits such as cochlear synaptopathy (CS) and inner-hair-cell (IHC) dysfunction can remain “hidden” when analyses focus primarily on thresholds. In animal models, moderate noise exposure can produce Temporary Threshold Shift (TTS) that often reflects synaptic loss without overt hair-cell damage or permanent threshold elevation. In chinchilla, carboplatin (CA) preferentially targets IHCs and their stereocilia, yet leaves behavioral/physiological thresholds largely unchanged. Nonetheless, quantitative analysis of suprathreshold waveform morphology (timing, amplitude, slope, inter-wave relationships) is commonly used to measure hidden hearing loss that differs from person to person and is highly time-consuming. We tested whether a compact deep-learning model can confidently detect these subtle differences in ABR waveform morphology to differentiate sensorineural hearing-loss subtypes.

Methods: Preclinical chinchilla datasets were used, representing three groups: normal hearing (Baseline), CS/TTS (noise exposure), and IHC dysfunction (carboplatin). ABRs were recorded using sub-dermal electrodes (vertex, mastoid, nose/ground), amplified 20,000×, band-pass filtered, and digitized at 48 kHz. For 2, 4, and 8-kHz stimuli, 500 repetitions per level were averaged; level-stacked traces were rendered as “waterfall” images that visualize time-domain morphology across levels. Images were standardized with consistent y-axis scaling.

We fine-tuned EfficientNet-B0 (ImageNet-initialized) for a two-stage pipeline: Stage 1 (gate) classified Normal vs Abnormal; Stage 2 assigned abnormal samples to CA (IHC dysfunction) or CS (synaptopathy). Training used targeted augmentations (minor rotation/contrast jitter), early stopping, and regularization to mitigate overfitting. Evaluation used sensitivity/precision and confusion matrices for both stages.

Results: Morphology encoded clinically relevant information beyond thresholds.

- Stage 1 (Normal vs Abnormal): high sensitivity and strong precision, indicating reliable detection of hidden loss from waveform morphology alone.
- Stage 2 (CA vs CS among Abnormal): High overall accuracy. Per-class metrics showed very high precision and sensitivity for IHC dysfunction, whereas strong sensitivity and decent precision for CS. Misclassifications occurred primarily between IHC dysfunction and CS, consistent with nuanced, overlapping suprathreshold morphology rather than gross differences.

Conclusions: A lightweight, morphology-aware pipeline based on EfficientNet-B0 can learn subtle suprathreshold patterns in ABR waterfall images. This enabled sensitive detection of hidden hearing loss and preliminary differentiation of across sensorineural hearing-loss subtypes (IHC dysfunction, CS) even when thresholds are unchanged. Ongoing work targets (i) expansion and balancing of the dataset across animals/conditions, (ii) refinement of image-construction choices (dynamic-range normalization, windowing tuned to physiologic landmarks). This approach has the potential to complement standard audiograms with an

automated analysis of ABR waveform morphology to provide individualized diagnostic insight.

SA180. POD-Vis: Interactive Hypothesis-Based Exploration and Visualization of Pure Tone Audiometry and Middle Ear Data in Clinical and Research Study Populations

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Background: Clinician researchers wanting to compare their own data with published studies or collaborators' datasets are faced with several challenges, including the need for data extraction and harmonization, study design, statistical support, data visualization and figure generation. We present POD-Vis (Probing Outcomes Data with Visual Analytics), a web-based data repository and analysis platform that eases the burden of performing these tasks. Originally agnostic with respect to both the clinical measurements and disease/condition of interest (e.g., Parkinson's disease, cancer), POD-Vis has been enhanced to account for audiometric data and underlying health conditions.

Methods: Audiometric datasets are harmonized and loaded into a MySQL database. Audiometry and middle ear variables are renamed as needed, and hearing threshold values processed to ensure consistent handling of masked and unmasked thresholds and no response (NR) values. Demographic data, health metrics, and other test results are handled similarly, with ICD-10 codes used to record diagnostic information. Once datasets are harmonized and loaded, a VueJS-based web application guides end-users through a scripted analysis workflow to combine datasets, select variables, and create and compare study groups (cohorts). Statistical analyses, including multivariable regression, can be performed using the Python SciPy and statsmodels packages. Data visualizations are displayed as interactive plots and charts based on Apache ECharts, Plotly, and D3.js. For pure tone threshold data specifically, a custom interactive audiogram has been developed. It displays cohort-level pure tone threshold data, with options for error bars and boxplots, extended high frequency data, and bone conduction data. This display can also overlay individual patient audiograms (e.g., for potential clinical use.)

Results: POD-Vis has been used to harmonize and analyze various datasets, including clinical, retrospective study, and general health survey data. Specifically, we have created an instance of POD-Vis for the HearShare Consortium. The consortium includes clinicians/scientists from Duke University, Massachusetts Eye and Ear, Medical University of South Carolina, University of Maryland Baltimore, University of Maryland College Park, the National Institute of Deafness and Other Communications Disorders clinical center, and Oregon Health Sciences University. For this consortium we have harmonized and loaded data from population-based studies such as the National Health and Nutrition Examination Survey (NHANES), the Framingham Heart Study, the Baltimore Longitudinal Study of Aging (BLSA), and the Hispanic Community Health Study (HCHS/SOL), plus clinical datasets from University of Maryland School of Medicine and Duke University. This comprises over

62,000 subjects with audiograms containing associated demographic and health information. POD-Vis has been deployed in both the Azure and Google Cloud environments, and pilot projects are ongoing with several collaborators.

Conclusions: POD-Vis is a data visualization and analysis platform that allows clinicians and researchers the ability to interact with large harmonized audiometric datasets and generate hypothesis-based explorations, without needing extensive prior experience in either data science or statistics.

SA181. The Sound of Safety: Why GLP Ototoxicity Testing Must Be Standard

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Background: Hearing loss affects approximately 1.5 billion people worldwide, with ototoxicity representing a leading cause of acquired hearing loss globally. The number of ototoxic drugs is estimated between 150-600, with over 200 marketed medications documented to cause ototoxicity. While FDA guidelines define otic assessments for drugs delivered directly to the ear, testing for systemically administered therapies remains optional, leaving critical regulatory gaps despite the potentially devastating consequences of auditory dysfunction

Methods: Current FDA regulations for Good Laboratory Practices (GLP) (21 CFR Part 58) govern nonclinical studies, with guidance for otic administration specifying auditory brainstem response (ABR) and cochleogram as primary endpoints. However, for systemically administered therapies, no dedicated guidance exists, leaving assessments to case-by-case evaluations and underscoring the need for transparent dialogue between sponsors and regulatory authorities. Study designs utilize 10 animals per sex per group with preferred species including rats (Wistars in Europe and Sprague Dawley in North America), CBA/CaJ mice (age-stable hearing characteristics), and Hartley guinea pigs (enhanced ototoxic sensitivity). Three doses are tested: low (pharmacological), intermediate, and high, with reversibility periods to assess effect permanence. Key assessment methods include ABR measuring cochlear and neural pathway functionality through synchronous neuronal discharge; and cochleogram providing histological assessment of hair cell loss across 4 rows. In addition, DPOAE, electroacoustic measure, assessing outer hair cell integrity through acoustic signals from cochlear epithelium can be performed. ; Clinical interpretation follows ASHA criteria defining significant hearing change as ≥ 20 dB decline at any frequency or ≥ 10 dB decline at two adjacent frequencies. Key components of GLP include an effective quality assurance program, proper management and staff training, adequate facilities and equipment, clear and documented procedures, and meticulous record-keeping to provide traceability and reproducibility of study results, with compliance ensured via regulatory agency audits and inspections.

Results: Preclinical data demonstrate that small threshold shifts (5-10 dB) without histopathological changes may represent normal test-retest variability, requiring integrated interpretation of ABR, DPOAE, and cochleogram findings. Therapy-specific study designs include: small molecules utilizing standard repeated-dose protocols with functional testing at baseline, interim, and terminal timepoints; gene therapy requiring specialized round window/cochleostomy injection with 3-6 month observation periods and biodistribution

studies; cell therapy employing local delivery approaches with engraftment monitoring and immune response evaluation; local delivery necessitating trans-tympanic or intracochlear routes demanding specialized surgical expertise

Conclusions: Ototoxicity assessment is established for local otic administration but remains optional for systemic dosing, reflecting on regulatory gaps. Comprehensive evaluation requires integration of electrophysiological and histological expertise and, for otic delivery, surgical proficiency. Given the devastating impact of auditory dysfunction and the absence of formal regulatory requirements, incorporating ototoxicity testing into standard GLP safety guidelines would enable earlier detection of auditory risk, prevent irreversible hearing loss, and strengthen the evidence base for therapeutic decision-making.

SA182. Adaptive Sampling of Dpoae Level Maps and Their Relation to Conductive Hearing Loss and Ear Canal Calibration

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Background: Distortion-product otoacoustic emission (DPOAE) measurements are strongly influenced by middle-ear transmission properties, thus, making conductive hearing loss a critical factor in clinical assessments. Complete characterization of the L1, L2-parameter space, referred to as DPOAE level-maps (LMs), allows for distinguishing between linear and non-linear losses, provided that forward and reverse transmission losses are closely correlated (approximately 1:1). The underlying theory asserts that anterograde conductive loss at f₁ and f₂ is reflected in a correspondent shift of the LM in the L₁, L₂-plane, and that retrograde loss at f DP is reflected in a correspondent reduction of the semilogarithmic slope along the LM ridge. Here, we show in (1) data from patients with suspected otosclerosis, and (2) data from different ear-canal calibration methods, that the underlying theory is largely confirmed by experiments.

Methods: DPOAE level maps were obtained from two groups: (1) 10 patients with conductive hearing loss (CHL) in at least one ear, based on air-bone gap (ABG) measurements, and (2) 11 normal-hearing (NH) subjects who underwent two sessions with shallow and deep probe insertions and four calibration methods (IPL, FPL, +EPL). The frequency range examined was 1–6 kHz for CHL patients and 1–14 kHz for NH subjects. For the ear-canal calibration analysis, inter-session changes in DPOAE LM parameters for the IPL, FPL, and IPL+EPL methods were correlated with inter-session variations in speaker calibration transfer functions (SPL) between the shallow and deep probe insertions.

Results: In conductive-loss patients, the change in LM ridge slope significantly correlated with ABGs at f DP, with an SDresidual of 4 dB (R²=0.56, N=39). The offset of the LM towards higher L1 levels also correlated highly significantly, but with a slightly higher SDresidual of 5 dB.

In the calibration study, inter-session changes in the calibration transfer function at f₁ significantly correlated with the shift of the optimal L1 value toward higher L1, with an SDresidual of 4.5 dB (R²=0.91, slope a=0.96). Similarly, inter-session changes in the calibration transfer function at f DP correlated significantly with the decrease of the semilogarithmic slope of the LM ridge, s. with a SDresidual of 3 dB (R²=0.43, slope a=0.95).

The DPOAE LM parameter L2,EDPT correlated with total hearing loss (conductive plus cochlear loss). This was verified using data from 9 ears (5 patients) with cochlear hearing loss from an independent study. The correlation between L2,EDPT and Békésy tracking audiometry from NH and CHL group is highly significant and yields an SDresidual of 6.8 dB ($R^2=0.61$, slope $a=0.96$) consistent with previous findings.

Conclusions: Accumulated hearing loss from conductive loss (whether pathological or due to calibration changes) and cochlear amplification loss (which behaves nonlinearly) can be differentiated by analyzing three key parameters of the DPOAE LM, namely L1,EDPT, L2,EDPT, and s.

SA183. Impact of Anesthesia on Otoacoustic Emissions and Blood Oxygen Saturation in Rats

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Background: Distortion-product (DPOAEs) and stimulus-frequency otoacoustic emissions (SFOAEs) are both considered as early, objective biomarker of subclinical hearing loss with outer hair cell (OHC) dysfunction receiving increasing attention in age-related hearing loss (ARHL), speech discrimination and early onset dementia. Preclinically OAE measurements are typically performed under deep anesthesia, mostly to complement ABRs. In our recent studies with extensive OAE measurements over 45 min utilizing a Fentanyl-based anesthetic regimen, we observed instability and inconsistency in the measurement amplitudes associated with low blood oxygen saturation (SpO₂). Here we report a comparison between Fentanyl and the Zoletil (Zolazepam-Tiletamine)-Xylazine regimens on OAEs, including a systematic monitoring of SpO₂ and DPOAE.

Methods: Ten 9 month-old male Wistar rats were monitored for SpO₂ and fast DPOAEs during two 60 min long recording sessions, 2 weeks apart. Anesthesia was successively induced using i.p. administration of either Zoletil/Xylazine 20-40/3.5-5 mg/kg or Fentanyl/Midazolam/Medetomidine 0.005/2.0/0.15 mg/kg. Unilateral audiometric characterization was performed in a soundproof chamber including swept-tone stimulus OAEs [4-42] kHz with high and low level DPOAEs, SFOAEs and ABR [8-32] kHz. The monitoring was performed every 5 min from 15 to 60 min of anesthesia. Lastly supplementary oxygen flow was added in the end of the OAE session to allow a final DPOAE measurement under 98% O₂.

Results: After 15 min of anesthesia the SpO₂ from both groups dropped to 88-89%. From 25 min the Fentanyl group was as low as 83 % O₂ whereas the Zoletil group maintained close to 86-87 % O₂ until 60 min. Low level DPOAE and SFOAE amplitudes from the Zoletil animals were markedly higher across the entire frequency range, with differences increasing from low to high frequencies, and an average 7 dB improvement on DPOAEs between [13.7-36] kHz and 9 dB on SFOAEs between [6.5-27] kHz. The average difference on high level DPOAE amplitudes were not as marked. Comparing the fast DPOAE monitoring before and after the supply of oxygen flow, showed an initially 5 dB higher output in the Zoletil group with no effect of supplementary oxygen, whereas supplementary oxygen in the Fentanyl group lead to DPOAE amplitudes matching the Zoletil group for most of the tested

frequencies. ABR thresholds showed only 0.3 to 3.9 dB difference across frequencies between anesthetic regimens.

Conclusions: These data suggest that DPOAEs and SFOAEs are highly sensitive to blood oxygen saturation and middle ear transmission. The Zoletil-Xylazine mixture seems better suited for this aging model. The choice of the anesthetics appears to have a significant, direct impact on the amplitude and the stability of these measurements and should be carefully determined for each animal model, perhaps depending on the sex and the age of the species, to facilitate analysis and comparison over time and across experimental groups.

SA184. Probes and Paradigms: Serial Observations of Extended Bandwidth Distortion Product Otoacoustic Emissions (DPOAEs) in Healthy Adults

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Background: Basal cochlear regions associated with higher frequencies of hearing are selectively damaged by factors such as aging and therapeutic treatments known to cause hearing loss. Identifying the tools to best reflect and identify this damage across the lifespan is critical to the identification and clinical management of patients. One such tool, distortion-product otoacoustic emissions (DPOAEs), has the potential to identify cochlear damage that has not yet been observed on the audiogram at both conventional and extended high frequencies (EHF; > 8 kHz).

The value of testing EHF-DPOAEs for earliest detection of cochlear dysfunction is well established; however, it is not standard clinical practice. This is in part due to challenges associated with complex ear canal acoustics in the EHF range and availability of transducers optimized for EHF assessments. While the reliability and repeatability of DPOAE measures at conventional frequencies obtained using clinical tools have been explored, comparable evidence is lacking for EHF stimuli. Until recently, commercially available DPOAE tools with the potential for expanded EHF stimuli (>=10 kHz) were not readily accessible for clinical translation. Thus, this study aims to characterize test-retest variability of a DPOAE test battery across an expanded frequency bandwidth in healthy participants for serial measurements and to further elucidate what constitutes a clinically meaningful change.

Methods: This study (NCT01629108) characterizes and compares serial measurements of standard (up to 8 kHz) and EHF (up to 16 kHz) DPOAE amplitudes from the same ear using four different probes across two systems (Interacoustics and Intelligent Hearing Systems). Testing was completed in each ear over three test sessions 2-3 weeks apart.

Participants (n=10; M/F=5/5; age range 19-63 years) completed a comprehensive audiological test battery in each ear including but not limited to hearing thresholds (up to 20 kHz), middle ear measures, as well as standard and EHF DPOAE measures. Inter-test and intra-test variability was determined for each probe and across probes when possible.

Results: Preliminary DPOAE outcomes are similar across sessions and equipment, with increased variability above 4 kHz. Stability of measures based on probe and paradigm (including discrete- versus swept-frequency) will be presented. Proposed clinical monitoring parameters and navigation of technical barriers to using EHF stimuli to enhance clinical utility will be discussed.

Conclusions: Preliminary findings support favorable test-retest variability across EHF-DPOAE probes investigated and the potential for enhancement of serial measurements for optimal clinical translation based on study outcomes. Future monitoring strategies and clinical applications of serial measurements will be discussed.

This work was supported by the Division of Intramural Research at the National Institute on Deafness and Other Communication Disorders (ZIA DC000064).

SA185. Development of a Ferret Model for Investigation of the Neural Correlates of Binaural Fusion of Vowel Sounds

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Background: Human listeners with hearing loss often experience abnormally broad binaural fusion, the perception of two spatially separated sounds with pitch differing by as much as 1-4 octaves as a single object (Reiss et al., 2017). Broad binaural fusion is associated with difficulties segregating voices and understanding speech in noise (Oh et al., 2019). The goal of this study was to develop a method of measuring binaural fusion behaviorally in an animal model and determine the neural correlates. Previous human behavioral studies have shown that two dichotically presented vowels are sometimes fused and perceived as a third vowel, distinct from either of the component vowels (Reiss and Molis, 2021). We have developed a go/no-go behavior to measure this fusion percept in ferrets.

Methods: Vowel fusion was measured in 10 normal hearing (NH) human subject listeners and 2 normal hearing ferrets. Synthetic vowels with fundamental frequencies (F0) 106 Hz and 151 Hz were used. The stimuli were presented through loudspeakers angled +/-60 degrees in the azimuth. Both parties were first trained to report the identity of a single vowel, either in a 6 AFC task (humans) or a go/no-go task (ferrets). During the experimental task, subjects were introduced to double vowel pairings, differing in vowel identity, and presented at either the same or different F0s. Double vowel presentations were interleaved with previously experienced single vowel presentations. Human subjects reported the one or two vowel(s) they heard during a trial through 6 options on a touch screen, with perception of a single vowel indicating fusion. Ferrets were trained to respond to indicate perception of AE or EH vowels (go) or OO, EE or AW vowels (no-go), with the perception of go stimuli for an AH-EE double vowel pair indicating fusion.

Results: Human behavioral data showed that double vowel pairs with the same fundamental frequency were often perceived as a single vowel, resembling the spectral average of the constituent vowels, consistent with previous experiments using stimulation over headphones. Ferret behavioral data show evidence of spectral averaging in line with the human subject data. Preliminary findings from neurophysiological recordings in the auditory cortex of ferrets suggest that neural responses to the fused double vowels are similar to those of the single vowel percept reported behaviorally.

Conclusions: Comparison of vowel fusion between human subjects and the ferret model suggests that both groups experience similar binaural fusion. Ongoing neurophysiological recordings will describe neural mechanisms producing binaural fusion and may provide insight into mechanisms and treatment of abnormal fusion following hearing loss.

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SA186. Cortical Projection-Specific Processing of Infant Vocalizations for Mouse Maternal Behavior

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Background: How does the brain derive meaning from social vocalizations and drive behavior in response? Vocalizations are complex stimuli that require integration across multiple time-scales and appropriate contextualization. Top-down modulation of feed-forward auditory processing is likely needed for these computations. To test this we took advantage of a robust mouse behavior, in which parental animals search for lost pups in response to infant ultrasonic vocalizations (USVs). Experienced moms are experts at this behavior, but nulliparous females can learn to respond to infant distress calls after cohousing with lactating moms and litters, and emergence of pup retrieval behavior is correlated with oxytocin-dependent plasticity in the left auditory cortex (Marlin et al. 2015, Schiavo et al. 2020).

Results: We asked whether projections from auditory cortex to subcortical areas are important for pup retrieval behavior in experienced maternal mice by chemogenetically silencing activity in left auditory cortex layer 5 during retrieval. Pup retrieval was reduced after CNO treatment vs vehicle control sessions (N=6 mice, $p < 0.05$). Silencing only neurons projecting to inferior colliculus led to a similar decrease (N=6, $p < 0.05$), while silencing only neurons projecting to the tail of striatum had no effect (N=6, $p > 0.05$), indicating that corticocollicular (CC) projections are particularly critical for linking perception to behavior in this context. We next used 2-photon Ca²⁺ imaging in awake head-fixed mice to compare USV responses of corticostriatal (CS) and CC neurons. CC neurons in expert retrievers (N=4 mice) had sustained increases in activity during USVs compared to pure tones, while activity was similar during USV and tone presentation in CS neurons (N=3 mice). This was corroborated with in vivo whole-cell recordings from optotagged projection neurons.

To examine whether sustained activity in corticofugal neurons develops with experience, we tracked activity in CC and CS neurons with 2-photon imaging over several days of co-housing. Robust responses to USVs were present on each day; however, responses were variable across neurons and days. In CC neurons, delayed and sustained responses to USVs increased over experience with pups, which may reflect upregulated activity in recurrent auditory circuits.

To examine how cortical projections modulate activity across auditory areas with precision for spike timing, we performed simultaneous silicon probe recordings from auditory cortex, inferior colliculus, and thalamus (N=6 mice). Subpopulations of neurons in each area integrated over sequences of call syllables. To test whether cortical feedback facilitates temporal integration, we optogenetically silenced corticofugal projections, which altered the timing of call-evoked spikes in inferior colliculus.

Conclusions: We found that corticofugal projections to the auditory midbrain are essential for vocalization-guided maternal behavior, and that neurons in this pathway integrate acoustic cues over behaviorally relevant timescales. Optogenetic inactivation further revealed that vocalization-evoked activity in the midbrain depends on top-down modulation rather than simple feedforward processing.

SA187. Auditory Cortex Function Can Be Characterized by Recurring Spontaneous Activation Patterns

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Background: Determining the functional organization of the auditory cortex (AC) has been difficult with conventional task-based approaches due to the broad responsiveness of auditory subregions to various acoustic properties. Moreover, most studies have assumed that the functional organization of AC is fixed, even though whole-brain research has shown that brain function is organized into constantly changing functional networks. These functional networks can be estimated from functional magnetic resonance imaging (fMRI) data by identifying coactivation patterns, groups of brain areas that are activated at the same time, under the assumption that simultaneous activation reflects functional cooperation. We hypothesized that coactivation patterns similar to those identified at the whole-brain level could also be reliably identified locally within AC. We further anticipated that the AC patterns identified during the resting state would represent fundamental functional processing modes, showing strong spatial similarity to patterns identified during auditory and audiovisual stimulation.

Methods: The AC patterns were estimated from high-resolution 7T fMRI data of 30 healthy volunteers, each of whom participated in three 2-hour fMRI sessions. The generalizability of the AC patterns was tested using the Human Connectome Project 7T fMRI dataset (N = 177). In the coactivation pattern analysis, individual fMRI volumes (time points) were clustered across participants using k-means based on their spatial similarity, and template coactivation patterns were generated by averaging the fMRI volumes within each cluster. Thereafter, within each participant, each of the fMRI volumes was classified into one of these templates based on spatial similarity. The coactivation patterns were compared between and within participants and datasets based on their spatial similarity and occurrence rate. An eight-pattern solution was selected for closer examination based on its high reproducibility values, which were 0.86 for the occurrence rates and 0.79 for the spatial topography.

Results: Dynamic AC patterns successfully captured interindividual variability, as indicated by significantly higher variability between than within individuals for the AC pattern occurrence rates and spatial topographies. The coactivation patterns shared similarities between resting-state and auditory-task data, as indicated by the group-level similarity of 0.84 and individual-level similarity of 0.71 in the spatial topographies. Furthermore, the occurrence rates of AC patterns identified in the task data, using pattern templates derived from resting-state data, correlated with specific task contrast regressors.

Conclusions: Our results indicate that the AC function can be characterized by a set of constantly varying coactivation patterns, presumably reflecting brain networks, that are consistently observed during resting state and auditory stimulation, and that get synchronized with auditory input. These findings enhance our understanding of the relationship between spontaneous and stimulus-driven activity in the AC and support the development of more time-efficient paradigms for studying its functional organization.

SA188. A Narrow Path for Long-Term Potentiation of Cortical Inhibitory Circuits

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Background: Following sensorineural hearing loss (SNHL), weakened inhibition fails to stabilize excitatory pyramidal neurons (PyrNs), leading to hyper-responsivity, hyperactivity, and hypersynchrony. A recent study from our lab has shown that PyrN hyperactivity and loudness hyperacusis can be sustainably reversed in mice with noise-induced SNHL via a single brief bout of 40-Hz activation of parvalbumin-expressing cortical inhibitory neurons (PVNs). Here, we addressed whether sustained dampening of PyrN activity is unique to PVN activation or whether it might also result from 40-Hz activation of another cardinal class of cortical inhibitory neuron: dendrite targeting somatostatin_{x0002}-expressing interneurons (SOM) or through non-specific activation of all cortical inhibitory neurons.

Methods: High-density translaminar single-unit recordings were made in the high-frequency regions of the primary auditory cortex of awake, head-fixed mice before and after 16 min of 40-Hz optogenetic activation of PVN, SOM, or pan-inhibitory activation in VGAT-ChR2-EYFP mice (N = 6/6/2 mice; 90/70/60 sound-responsive regular spiking units). Sustained changes in excitability were assessed by measuring changes in the efficacy of optogenetically induced feedforward inhibition of putative PyrNs 30- and 60-minutes following 40-Hz activation compared to pre-activation baseline recordings.

Results: We found that 40-Hz activation of PVN-, SOM-, or VGAT-expressing inhibitory neurons elicited a robust gamma rhythm in the cortical local field potential and entrained photo-tagged single units spiking at 40 Hz. Whereas VGAT and PVN activation entrained the spike phase of regular spiking (RS) and fast spiking (FS) units in the canonical pyramidal-interneuron gamma (PING) rhythm, 40-Hz SOM activation did not. One hour after the cessation of 40-Hz PVN activation, PVN-mediated inhibition of RS units spiking was significantly enhanced, and sound-evoked RS spiking was significantly reduced without any change in spontaneous firing rate. Conversely, one hour after 40-Hz activation of SOM or VGAT neurons, the spontaneous firing rate was elevated without any change in feedforward RS unit inhibition or sound-evoked RS unit spike rate.

Conclusions: We found that 40-Hz activation of any cortical inhibitory neuron type elicited a robust gamma rhythm but only PVN or pan-inhibitory activation generated the characteristic FS-RS spiking dyad—a hallmark of the PING rhythm. Long-term potentiation of cortical inhibition was only observed with 40-Hz PVN activation, with no change following VGAT or SOM activation. When considered alongside our prior study showing that inhibitory potentiation was specific to 40-Hz PVN activation, our findings highlight a remarkable specificity for dampening hyperactive cortical circuits via temporally patterned activation of inhibitory circuits. That long-term inhibitory potentiation is effective at reversing hyperacusis in mice with irreversible cochlear sensorineural damage identifies new therapeutic avenues for treating suprathreshold hearing disorders like tinnitus and hyperacusis. That long-term inhibitory potentiation is specific to PVNs at specific activation rates may explain why systemic administration of GABA agonists has been ineffective as treatments for tinnitus and hyperacusis.

SA189. Robustness to Noise Reveals Cross-Culturally Consistent Properties of Pitch Perception for Harmonic and Inharmonic Sounds

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Background: Pitch is a building block of speech and music, but the extent to which pitch perception abilities are shared across cultures is unclear. Evidence from Western participants suggests that pitch perception relies on multiple representations. For instance, harmonic tones are easier to discriminate in noise than inharmonic tones (that lack a clear fundamental frequency) despite comparable discrimination in quiet, suggesting that different representations are used in noise and quiet. However, parallel work comparing participants in the USA and Tsimane, a group of hunter-agriculturalists living in the Bolivian Amazon, has suggested that some pitch-related behavior varies in at least some respects across populations. When asked to reproduce brief melodies by singing, USA listeners tend to sing back notes where the f_0 matches the stimulus frequency ('pitch matching'), as well as notes that are related to the stimulus notes by integer numbers of octaves ('chroma matching', i.e., frequency ratios that are powers of two), suggesting that notes related by octave ratios are treated as musically equivalent by USA listeners. By contrast, Tsimane' listeners exhibit no such tendency to chroma match, and a reduced tendency to pitch match. This set of findings left open the extent to which pitch perception varies in other respects across cultures. We sought to resolve this issue by assessing other aspects of pitch perception cross-culturally.

Methods: USA and Tsimane' participants heard two-note melodies and reproduced the melody by singing. Tones were either harmonic or inharmonic and were presented in noise at either high or low signal-to-noise ratios (SNRs).

Results: USA and Tsimane' participants both exhibited two characteristics of pitch perception previously seen in US listeners: the direction of pitch changes could be reproduced with equal accuracy for harmonic and inharmonic tones at high SNR, but was better for harmonic than inharmonic tones at low SNR. However, replicating prior results, Tsimane' did not exhibit the tendency of US participants to match the absolute pitch or chroma of the stimulus notes.

Conclusions: The basic structure of pitch perception appears to be shared across cultures despite other differences in pitch-related behavior; both US and Tsimane showed signatures of having two distinct pitch mechanisms, and to use the two mechanisms in the same way, despite differences in pitch and chroma matching tendencies during singing.

SA190. Multiple Statistical Integration Windows in Auditory Perception

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Background: Sound textures, as arise from swarming insects or falling rain, are believed to be represented with time-averaged statistics. Texture statistics show signs of being averaged over an integration window spanning multiple seconds. However, many different statistics are needed to account for texture perception. It remains unclear whether there is a single global integration window for all statistics, or whether integration windows vary across statistics.

We investigated the extent of time-averaging for individual classes of statistics from the auditory texture model of McDermott and Simoncelli (2011). We divided the statistics into fourteen classes spanning different audio and modulation frequencies, and marginal as well as correlation statistics.

Methods: We conducted an adaptive threshold experiment in which listeners judged which of two synthetic sound textures was most similar to a reference texture. The two textures on a trial were designed to differ in one of the fourteen classes of statistics. We measured discrimination thresholds for stimuli of different durations, ranging from 20ms to 10s.

Results: For each statistic class we tested, texture discrimination thresholds decreased with stimulus duration and then leveled off, potentially corresponding to the duration at which the stimulus exceeded the time-averaging window used to estimate the relevant class of statistics. The performance asymptote occurred at different durations for different statistics, and a model with statistic-specific integration windows provided the best account of the results.

Conclusions: The results suggest the extent of time-averaging varies across texture statistic classes, revealing a continuum of statistical integration windows in auditory perception.

SA191. Modeling Musical Pitch Perception With Mild to Moderate Sensorineural Hearing Loss

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Background: Pitch is a key auditory feature for music perception and appreciation, providing the basis for melodies (from isolated pitches) and harmonies (from combinations of pitches). Mild to moderate sensorineural hearing loss (HL) degrades spectrotemporal cues and adversely affects perception of single pitches, but less is known about how HL affects multiple-pitch perception. In computational models of hearing, the perception of single pitches has been well studied, but fewer studies have investigated multiple-pitch perception. Our study tested listeners with and without HL on pitch perception tasks ranging from simple pitch discrimination to chord perception in musical context. These data allow us to measure the impact of HL on multiple pitch perception and compare it against established effects on perception of single pitches. We also developed novel stimuli based on a physiologically grounded computational model of the auditory system, to simulate effects of hearing loss on perception of single- and multiple-pitch stimuli.

Methods: In the behavioral study, we measured pitch discrimination thresholds in listeners with mild to moderate HL for four pitch perception tasks involving single-pitch stimuli and multiple-pitch combinations. Specifically, listeners were instructed to (1) compare two sounds and chose the sound with the higher pitch, (2) determine whether a target pitch in a chord is higher or lower than an isolated reference pitch, (3) discriminate major from minor chords, and (4) rate chord sequences for expectation. In the modeling study, we created rate-place metamers of pitch stimuli using the average rate output of a well-known auditory model (Zilany, Bruce, and Carney, 2014). Model parameters were adjusted to include HL that matched the profiles of listeners with HL in the behavioral study. The resulting stimuli were then tested on the same battery of behavioral tasks with NH and HL listeners, to measure effects of HL on pitch cues carried in the rate-place code.

Results: Preliminary data suggest that HL results in mild deficits in single-pitch perception (task 1), with severe deficits for discriminating a target pitch masked by concurrent pitches (task 2). However, some HL listeners retain ability to discriminate chords (task 3) and perceive chords in musical context (task 4). Overall, modeling results suggest that degraded rate-place cues can account for worsened pitch perception in all tasks.

Conclusions: Our findings suggest that although mild-to-moderate HL impairs discrimination of a single pitch in a mixture of pitches, ability to perceive chords and harmony is relatively spared, suggesting that higher-level processes may compensate for degraded sensory input. Potential mechanisms underlying perception of chords and harmony may differ from single pitch perception mechanisms and warrant further study.

SA192. Mid-Bandwidth Loudness Depression for Sinusoidally Amplitude-Modulated Tones

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Background: Previous research has shown that the loudness of narrowband noise varies with its bandwidth. As bandwidth increases while fixing the center frequency and overall sound pressure level, the perceived loudness of the narrowband noise initially decreases, falling below that of the pure tone, and then increases again at higher bandwidths. This non-monotonic relationship, characterized by a reduction in loudness at mid-bandwidths relative to pure tones, is referred to as mid-bandwidth loudness depression. The present study investigates this phenomenon across a wide range of stimulus levels and frequencies using sinusoidally amplitude-modulated (SAM) tones to precisely control envelope fluctuations associated with bandwidth variations.

Methods: Twenty participants with normal audiograms were tested monaurally using insert earphones in a sound-treated booth. A rapid quick-Categorical-Loudness-Scaling (qCLS) procedure was used to assess loudness profile across five levels (20, 40, 60, 80, and 100 phons) and frequencies ranging from 250 to 8000 Hz. Stimuli were SAM tones with bandwidths defined by the spacing between upper and lower sidebands: 0 (unmodulated pure tones), $\frac{1}{4}$ octave, 1 octave, 40 Hz, and 200 Hz. The modulation depth was fixed at 100% and the modulation rate was half of the stimulus bandwidth. On each trial, the participant rated the loudness of the presented SAM tone using 11 categories ranging from “Can’t Hear”, “Very Soft”, to “Very Loud” and “Too Loud”. The qCLS procedure adaptively updated a parametric model of the participant’s loudness profile and optimized the frequency and level of the stimulus for the subsequent trial.

Results: The findings confirmed the presence of mid-bandwidth loudness depression in normal-hearing listeners. The effect was most pronounced at lower sound levels and higher frequencies, with the largest differences in equal-loudness contours exceeding 10 dB. The specific bandwidth at which the greatest loudness depression occurred varied depending on both stimulus level and frequency.

Conclusions: These results replicate and extend previous findings, demonstrating that mid-bandwidth loudness depression occurs across a broader stimulus space than previously studied. The findings underscore the critical role of temporal envelope fluctuations in shaping loudness perception.

SA193. Cues in Auditory Discrimination and Identification of Time of Day for Natural Soundscapes

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Background: Previous work suggests that human listeners can detect structure in natural soundscapes, and that spectral information is involved in the discrimination of these ecological scenes (e.g., Apoux et al., 2023). The present study extended this work to the perception of time of day, and assessed which acoustic cues (fine spectral detail, temporal envelope, temporal fine structure) mediate performance.

Methods: Normal-hearing adults were asked to perform time of day identification and discrimination for four broadly defined moments: Dawn, Midday, Dusk, Night. Identification used a single-interval 4AFC task; discrimination used a three-interval oddity task. Soundscapes were scenes recorded in three natural habitats (two temperate forests, one tropical) with minimal anthropogenic noise. To probe cue use, scenes were processed with a noise vocoder to alter temporal fine structure (TFS) cues and selectively degrade spectral or temporal information by manipulating the number of bands and the cutoff of the envelope filter.

Results: Participants showed poor identification of time of day, even when provided with feedback and after several hours of familiarization. In contrast, soundscape discrimination based solely on the time of recording was always very reliable. In fact, all the manipulations performed to degrade the signals generally resulted in limited effects. Overall, discrimination was especially robust to manipulations of envelope and TFS. Selective degradations of spectral information reduced performance in some extreme conditions, suggesting that decisions relied on gross spectral cues (e.g., ambient-noise). The impact of spectral degradation varied by habitat, indicating that the effective spectral resolution used by listeners depends on scene statistics specific to each forest type.

Conclusions: Human listeners struggled to identify time of day based on 1-s samples of natural soundscapes. However, they could discriminate these short samples very reliably. This was found to be true for soundscapes recorded from three different habitats. The soundscape recorded closer to the equator led to the best discrimination performance, perhaps partly because of greater biodiversity. The identification-discrimination dichotomy suggests that while human listeners are sensitive to acoustic variations related to differences in time of day, they cannot consistently label these variations.

Despite inconclusive data, it is suggested that biophony – the collective sound produced by animal vocalizations – did not play a critical role in time of day discrimination, probably because this cue was often inconsistent in the short duration of the samples used here.

Consistent with previous work, the present results suggest that human listeners can use spectral and temporal cues, but that they base their decisions primarily on spectral cues, the resolution of which depends on the habitat. However, none of these cues were essential for performance of the task.

Future work should test individuals with hearing loss and evaluate device algorithms that maintain spectral integrity in complex, naturalistic scenes.

SA194. Source-Location Binding Errors in Auditory Scene Perception

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Background: Where was my phone ringing from? Humans have some ability to discern both what sounds are present in a scene and where sounds are located. However, little is known about how identity and location are jointly represented ("bound" together), especially in multi-source scenes. In particular, it remains unclear whether humans make auditory binding errors in which source identities and locations are individually represented accurately but then mis-associated.

Methods: We probed for binding errors using an auditory search task. Participants listened to auditory scenes composed of between 1 and 5 natural sounds (e.g., applause, fireworks, laughter). Sounds were played from a speaker array, with sources being presented a minimum of 20 degrees apart. Participants were cued with a target sound either before or after the scene and were instructed to report the location of the target. We measured binding errors as errors in which the reported source location was close to the location of one of the other sources in the scene. To assess whether these errors could be explained without binding-specific failures, we simulated a null model that made errors either due to noise about the true source location or guessing (as if the target source was not heard).

Results: On average, localization error increased with the number of sources in the scene, and was higher in the post-cue compared to the pre-cue condition. In the post-cue conditions with 4 or 5 sources, participants made substantially more binding errors than the null model, suggesting that some such errors are caused by a failure to correctly associate sound identity with spatial location. By contrast, binding errors in the pre-cue conditions were no greater than predicted by the null model.

Conclusions: Humans maintain a joint representation of sound identity and spatial location for auditory scenes, but this representation becomes susceptible to errors in large scenes. In conditions without directed attention, some such errors appear to be due to binding failures, suggesting a limitation on auditory feature integration.

SA195. How Timbre and Harmonicity Affect Voice Perception in Polyphonic Music: A Comparison of Voice Enumerability and Discrimination Across Listener Groups

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Background: Listeners use multiple cues for stream segregation in speech and music. While well studied in speech, these cues are less understood in music. Previous work has shown that inharmonicity impairs listeners' ability to count the number of voices and hear out individual voices in polyphonic music, when all the voices share the same timbre. Here we tested whether differences in timbre improve performance and mitigate the detrimental effects of within-voice inharmonicity. Performance was tested in both hearing-impaired (HI) listeners and age-matched listeners with normal hearing (aNH).

Methods: Participants listened to short polyphonic instrumental passages with voices that were either of the same timbre, similar timbre (i.e., same family of instruments), or different timbres. The voices in each passage were either all harmonic or all inharmonic (with harmonics randomly jittered by $\pm 30\%$ of the fundamental frequency, F0). Two tasks were

tested: (1) voice counting, where the number of voices could be between 1 and 4, and (2) melody tracking, where listeners judged whether a probe tone matched the last note of a cued voice within a passage consisting of 2 to 4 voices.

Results: In the counting task, preliminary results confirm earlier studies, indicating that accuracy declines with increasing number of voices, and that inharmonicity degrades performance, particularly for small numbers of voices. Timbral differences between voices within each passage seem to improve performance, with early data suggesting improvements both for similar-timbre and different-timbre conditions in the aNH group, but only for the different-timbre condition in the HI group. In the melody tracking task, performance dropped overall with inharmonicity, with preliminary data suggesting a relatively weak trend toward benefits based on timbral differences for both listener groups.

Conclusions: Preliminary results suggest that timbral differences between voices may support stream segregation differently for aNH and HI listeners: whereas aNH listeners may benefit already from subtle timbral differences, HI listeners may require more salient differences. Overall performance in both tasks and in both groups decreased with increasing number of voices and with inharmonicity.

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SA196. Auditory Roughness Drives Subjective Aversion and Sustained Neural Synchronization

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Background: The ability to detect salient sounds and react rapidly is vital for survival. Among acoustic features, loudness (sound intensity) and roughness (fast amplitude modulations between 30–150 Hz) are particularly effective at capturing attention, as exploited by screams and alarm signals. Previous work has shown that roughness non-linearly enhances auditory aversion and accelerates reactions, possibly via sustained auditory steady-state responses (ASSRs) in non-classical auditory pathways linked to startle circuits. Since this reflex typically activates above 60 dB, we investigated whether aversive responses to roughness depend on sound level. We tested two hypotheses: (i) increasing sound level amplifies sensitivity to roughness, and (ii) heightened aversion to roughness reflects stronger ASSRs.

Methods: We measured behavioral (subjective aversion) and EEG responses in 29 normal-hearing participants exposed to click trains (10–250 Hz) at four intensities (40, 50, 60, 70 dB).

Results: Aversion increases linearly with sound level. Notably, the non-linear sensitivity to roughness emerges only at higher intensities, peaking around 40 Hz. EEG revealed robust ASSRs with maximal responses at 40 Hz. However, unlike aversion, 40 Hz ASSR amplitude increases linearly with sound level. Across participants, stronger roughness aversion correlates with larger ASSRs.

Conclusions: These findings show that roughness drives sustained neural synchronization and that sound level enhances roughness sensitivity at higher intensities. Entrainment around 40 Hz may explain why rough sounds are attention-capturing and supports the use of ASSRs as individual markers neural markers of salience sensitivity.

SA197. Auditory Processing of Acoustic Cues and Speech Recognition in Noise in Children With Mild to Moderate Hearing Loss

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Background: Sensorineural hearing loss (SNHL) is associated with reduced speech perception in noise, a difficulty thought to arise from impaired temporal and spectral resolution in addition to reduced audibility. Understanding how congenital mild-to-moderate SNHL affects auditory development in children is critical, as speech-in-noise perception underpins language learning. This study aimed to characterize auditory processing, speech-in-noise perception, and related cognitive skills in children with SNHL compared to age-matched peers with normal hearing (NH).

Methods: Twelve French-speaking children with mild-to-moderate bilateral SNHL (ages 4.5–10 years) completed a battery of auditory and cognitive tasks. Auditory measures included slow (4 Hz) and fast (20 Hz) amplitude modulation (AM) and frequency modulation (FM) detection, and spectral ripple discrimination (0.5 and 2 Ripple Per Octave). Speech-in-noise (SIN) perception was assessed using fricative consonant and disyllabic word identification tasks, each presented in stationary speech-shaped noise (SSN) and two-talker babble noise. Stimuli were amplified to compensate for audibility losses, with a maximum presentation of 85 dB SPL. Cognitive assessments included non-verbal working memory (Corsi blocks) and receptive vocabulary (PPVS). Performance was compared to a normative dataset of 66 age-matched children with NH, using z-scores to situate SNHL outcomes relative to the distribution of children with NH.

Results: Children with SNHL demonstrated AM detection thresholds comparable to NH peers, with some children performing slightly better. In contrast, FM detection thresholds were consistently elevated, especially at 20 Hz. Spectral resolution was also reduced, with a task almost unfeasible. For SIN perception, children with SNHL required substantially higher SNRs than NH peers to identify both consonants and words, across both SSN and babble conditions. Group with SNHL z-scores confirmed these observations with SIN identification and FM detection thresholds far outside the NH range. Vocabulary scores were reduced in nearly all children with SNHL, whereas non-verbal working memory seemed to be preserved. Preliminary correlation analyses revealed no significant associations between pure-tone thresholds and auditory or SIN outcomes, suggesting that audibility alone cannot account for the observed variability.

Conclusions: This preliminary study shows that children with mild-to-moderate SNHL present specific auditory processing deficits—namely in FM and spectral resolution—alongside preserved AM detection. These deficits translate into poorer SIN perception, regardless of target or masker, and are accompanied by reduced vocabulary knowledge. Importantly, the absence of a strong link between audibility and performance emphasizes the role of supra-threshold auditory and linguistic mechanisms in shaping communication outcomes. Clinically, these findings underscore the need for comprehensive assessments that extend beyond audiograms to include auditory processing and language evaluations. Future work with a larger sample will clarify developmental trajectories and inform rehabilitation strategies aimed at improving everyday communication in children with SNHL.

SA198. Listening Effort During Speech Perception in Noise: A Comparison of Binaural and Monaural Listening

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Background: Speech perception deficits are often overlooked in individuals with single sided deafness (SSD) because the “better ear” is assumed to compensate. Yet, SSD removes binaural benefits such as summation and spatial release from masking, and the individuals with SSD tend to have poorer academic performance and report psychosocial difficulties compared to binaural listeners. Additionally, binaural listeners exhibit a right ear advantage for speech, reflecting left-hemisphere language dominance. Since the side of deafness varies across SSD, here, we asked if unilateral hearing (as a proxy for SSD) differentially taxes speech perception in noise (SPIN) performance and attentional control.

Methods: Fifty-seven right-handed, normal hearing, native English-speaking adults aged 18-40 years were recruited using an online platform (Prolific). Participants completed a headphone check, and were tested using a custom, open source online psychophysical testing platform developed by us. Tests included speech perception in noise tests under informational masking (Matrix Sentences) and energetic masking (Modified Rhyme test) paradigms, and tests of distraction suppression (Auditory Stroop). Each participant completed these tests in a binaural condition, monaural-right condition, and monaural-left condition randomized in presentation order.

Results: Improved performance in binaural conditions compared to monaural conditions, and improved performance in the right ear compared to the left was observed for SPIN performance under informational masking, consistent with binaural and right ear advantages. This binaural advantage in behavioral performance was not present under energetic masking. However, participants exhibited slower reaction times in the monaural-left condition, consistent with increased listening effort. Auditory Stroop cost did not differ between binaural and monaural-left conditions but was larger in the monaural-right condition, indicating greater distraction suppression demand when relying on right ear input.

Conclusions: These findings from a unilateral-listening paradigm suggest that ear of input has a significant effect on SPIN. Under informational masking, right-ear listening confers an accuracy advantage over left-ear listening, while also exhibiting greater Stroop costs, suggesting a trade-off in attentional control demands, while energetic masking may reveal subtler effects in listening effort. Because participants self-reported normal hearing and testing was conducted online, results should be interpreted as simulated SSD, with ongoing studies testing the generalizability of these results in listeners with SSD. Clinically, quantifying listening effort alongside SPIN may inform counseling and intervention decisions and reveal hitherto hidden listening deficits in individuals with SSD.

SA199. Six1 and Eya1 Reprogram Postnatal Cochlear Supporting Cells Into Hair Cells

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Background: Mammalian cochleae fail to regenerate hair cells (HCs) after damage because postnatal supporting cells (SCs) lack regenerative capacity. We previously showed that Six1

and its cofactor Eya1 can induce nonsensory epithelial cells to differentiate into HCs in embryonic cochlear explants.

Methods: Using Sox2-CreER, we induced Six1 or Six1-Eya1 in SCs at postnatal day (P)5-P7 and analyzed cochleae 2 weeks later. To assess whether HC loss sensitizes SCs to reprogramming, we performed diphtheria toxin-mediated HC ablation at P7.

Results: In undamaged ears, Six1 alone converted a small percentage of SCs into Myo7a⁺ cells, whereas Six1-Eya1 together induced more SCs into Myo7a⁺ cells, indicating that Eya1 enhances the ability of Six1 in reprogramming SCs into HCs. In damaged cochlea, Myo7a⁺ cells induced by Six1 or Six1-Eya1 together were increased compared to those in the undamaged cochlea. EdU tracing indicated direct SC-to-HC conversion rather than proliferation-mediated replacement. Notably, a small subset of regenerated Myo7a⁺ cells induced by both Six1-Eya1 in the damaged cochlea was Sox2⁻/Prestin⁺, indicating maturation toward outer HCs.

Conclusions: These findings demonstrate that Six1-Eya1, promotes postnatal SC-to-HC reprogramming.

SA200. Early BMP4 Modulation Alters Electrophysiological Activity in Human iPSC-Derived Otic Organoids

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Background: Human induced pluripotent stem cell (hiPSC)-derived inner ear organoids offer a powerful model for investigating early otic development and hair cell function in vitro. While BMP4 is known to play a key role in ectodermal patterning, its dosage-sensitive influence on neurosensory specification and functional maturation in organoid systems remains incompletely characterized. Although low BMP4 levels favor neural ectoderm and high levels promote non-neural or epidermal fates, it is the intermediate BMP signaling window that is thought to best support otic induction. However, how such modulation impacts downstream electrophysiological output has not been fully explored.

This study evaluates the effects of graded BMP4 exposure during early ectodermal induction on the morphological, molecular, and functional development of hiPSC-derived otic organoids, with a particular focus on spontaneous extracellular activity assessed via multi-electrode array (MEA) platforms.

Methods: Organoids were generated using a stepwise differentiation protocol, in which BMP4 concentrations were varied during the first three days of culture. Samples were collected at multiple timepoints for imaging and immunofluorescent staining with epithelial and hair cell markers, including E-cadherin and Myosin VIIa. Between Days 75 and 100, organoids were transferred to MEA chips to record spontaneous spike activity, allowing comparison of firing frequency, burst organization, and network synchrony across conditions.

Results: Organoids exposed to intermediate BMP4 levels exhibited organized epithelial structures, strong E-cadherin localization, and a higher frequency of Myo7a-positive cells. These groups demonstrated robust and temporally structured MEA signals, including consistent spike trains and coordinated bursting across multiple electrodes. In contrast, both low and high BMP4 treatments produced irregular epithelial morphologies and either weak or asynchronous MEA signals. While lower BMP4 levels appeared to enhance general neural

excitability, they often lacked clear epithelial domains, suggesting an imbalance in neurosensory architecture. Excess BMP4 exposure reduced overall activity, likely due to lineage diversion away from both neural and otic fates.

Conclusions: Early modulation of BMP4 signaling exerts a lasting effect on the functional output of otic organoids. Optimizing BMP4 exposure supports balanced otic epithelial and neural specification, leading to more robust electrophysiological maturation. These findings advance our understanding of morphogen-guided inner ear development and contribute to the refinement of stem cell-based auditory disease models and therapeutic platforms.

SA201. Robust Hair Cell Regeneration out of LGR5+ Primary Cochlear Progenitor Cells Derived From Adult Mice

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Background: Supporting cells (SCs) in the cochlea give rise to hair cells (HC) in embryonic development. SCs in adult mice express the adult-tissue derived stem cell marker LGR5. Promoting hair cell (HC) regeneration using neonatal mouse models has been the focus of extensive research. Neonatal SCs have increased progenitor potential compared to adult and only a few studies (including ours) have shown survival of LGR5+ SCs after severe HC loss in adult mice. In mammals, there is no evidence for spontaneous HC regeneration in adulthood. However, three-dimensional cultures have allowed the expansion and experimentation of cochlear organoids. Here, we evaluated HC differentiation from cochlear organoids from primary LGR5+ progenitor cells derived from adult mice.

Methods: Adult (postnatal day 40-60) male and female *Lgr5-eGFP-IRES-creERT2* heterozygous normal hearing mice were used. Cochleas were harvested and digested to single cell suspension. Cells were filtered, mixed with Matrigel and 3D drops were made. Cells were grown on expansion medium (EM) for 10 days and differentiation medium (DM) for an extra 10 days. Organoids were fixed, permeabilized and processed for immunofluorescence confocal microscopy. We compared the published EM-M and DM-M (McLean et al 2017, doi: 10.1016/j.celrep.2017.01.066) and EM-X and DM-X (Xia, et al 2023, doi: 10.1016/j.stemcr.2022.11.024), to optimized media developed in our lab (OptiEM and OptiDM). The OptiEM contained surrogate wnt and r-spondin, to activate Wnt signalling; and the OptiDM contained recombinant sonic hedgehog and LY411575, to inhibit Notch signalling.

Results: We observed similar number of cochlear organoids 10 days after isolation in the different EM evaluated; however the size (area) of organoids was significantly larger in OptiEM. We found enhanced LGR5 expression in OptiEM compared to EM-M and EM-X. Importantly, in contrast to DM-M and DM-X, we observed robust expression of MYO7A in a structured row of cells located at the rim of the organoids, most of which had HC-like shape, in OptiDM. We are currently characterizing prestin expression, FM1-43 uptake, phalloidin staining and analyzing transcriptomic data in our newly developed culture conditions.

Conclusions: LGR5+ progenitor cells derived from adult normal hearing mice are able to produce cochlear organoids in different EMs. Enhanced LGR5 expression was observed in expansion conditions when culturing with surrogate wnt and r-spondin compared to published media containing CHIR99021 (EM-M and EM-X). Robust MYO7A expression,

indicative of sustained and organized HC generation out of LGR5⁺ progenitor cells, was achieved after activating sonic hedgehog and inhibiting Notch signalling. These results suggest that LGR5⁺ cochlear progenitor cells can be activated to promote HC regeneration even when derived from adult mice.

SA202. Clonal Dynamics Reveal Distinct Populations of Proliferative Cells in the Mouse Utricle Post Injury

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Background: The utricle is a vestibular sensory organ in the inner ear that plays a vital role in maintaining balance and spatial orientation. It contains specialized mechanoreceptors known as hair cells (HCs), which detect linear acceleration. In mammals, regeneration of HCs from interdigitated supporting cells (SCs) primarily occurs during early postnatal development. Following in vivo damage, the utricle exhibits a regenerative response in which SCs give rise to new HCs through two mechanisms: transdifferentiation and mitotic regeneration. The mitotic mechanism of regeneration is important for tissue homeostasis during injury. Whether there are intermediate cell states with a hierarchy of asymmetrical cell division and replenishment of existing cells in the mouse utricle post injury in vivo is currently unknown. Here, we investigate the clonal dynamics of mitotically regenerated cells following in vivo hair cell damage.

Methods: The molecular mechanisms governing lineage segregation of injury-activated proliferative cells remain poorly understood. To investigate this, we examined cellular heterogeneity within the injured utricle using an established in vivo hair cell ablation model (Pou4f3DTR), combined with two different inducible Cre recombinase lines and a mitotic cell division marker. Hair cell damage was induced at postnatal day 1 to selectively ablate hair cells. To study mitotic regeneration, we employed a triple transgenic model (Pou4f3DTR; Ki67CreERT2; Rosa26mTmG) for fate mapping of proliferating cells. Single-cell RNA sequencing (scRNA-seq) was performed following lineage tracing to interrogate clonal cells.

Results: Adult utricles in the damage model exhibit significant hair cell loss compared to undamaged wild-type controls. Cell proliferation, as indicated by EdU incorporation, is elevated in both the hair cell and supporting cell layers relative to wild-type utricles. Histological analysis using our triple transgenic model (Pou4f3DTR; Ki67CreERT2; Rosa26mTmG) reveals that cells undergoing mitosis undergo a permanent color switch to EGFP. Quantitative assessment of clonal fate and proliferation dynamics identifies distinct clusters of proliferative cells organized in a hierarchical structure, involving both transient amplifying and long-term proliferative populations.

Conclusions: In mammalian utricles, where regenerative capacity is limited, clonal tracing studies using transgenic animal models have advanced our understanding of cell fate behavior and its regulation under injury conditions. Through these studies, we demonstrate that clonal dynamics may converge toward a critical state defined by characteristic clone sizes, offering valuable insights into the mechanisms governing clonal behavior in damaged utricles in mice.

SA203. GSK3 β –EGF–TGF β Crosstalk Drives Vestibular Supporting Cell Proliferation

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Background: The mammalian utricle displays limited spontaneous hair cell (HC) regeneration and proliferation. Wnt/ β -catenin pathway plays diverse roles in the inner ear, including supporting cell (SC) proliferation and HC differentiation in both the cochlear and vestibular organs. Previous studies showed that overexpression of β -catenin, the central mediator of canonical Wnt signaling, increases proliferation and HCs regeneration in the neonatal utricle. GSK3 β functions to phosphorylate β -catenin, rendering it unstable and thereby inactivating the Wnt/ β -catenin pathway. However, GSK3 β also acts on numerous other pathways, such as NF- κ B synthase and mTORC1 pathways. Previous research showed that a combination of factors (including GSK3 inhibitors) increased proliferation in the neonatal cochlea (McLean et. al., 2017) and utricle (Kozlowski et al., 2020), but whether this occurs through Wnt/ β -catenin signaling remains unclear. Here, we examined the mechanisms by which GSK3 inhibition regulate SCs proliferation in the neonatal mouse utricle.

Methods: Utricles were dissected from postnatal (P) 3 C57BL/6 wildtype mice in aseptic conditions. Whole organs were cultured in serum-free media with CHIR, EGF, bFGF, IGF-1, VPA, pVc, TGF β i, and EGFR inhibitor. Neomycin was added for 24 hours to induce HC loss, followed by 5 days of culture with EdU to label proliferating cells. Tissues were stained for EdU, Myosin7a, Sox2 and counterstained with DAPI. EdU+ Sox2+ SCs were quantified in the striolar and extrastriolar regions. Furthermore, utricles from UBCCreERT2/+; Ctnnb1^{fl(exon3)}/+ and UBCCreERT2/+; Ctnnb1^{flox/flox} mice were treated by 4-hydroxy-tamoxifen to ablate or stabilize beta-catenin.

Results: The combination of seven factors robustly increased EdU+ Sox2+ SCs in both the striolar and extrastriolar regions of the neomycin-damaged utricles. Removing CHIR or TGF β i from the cocktail completely abolished proliferation throughout the utricle. While the absence of EGF or VPA from the cocktail significantly reduced proliferation in the extrastriola, removing bFGF, IGF-1, pVc had no significant effects, suggesting that these three factors are dispensable. Co-treatment with CHIR and TGF β i induced proliferation in the striola, whereas 4 factors (CHIR, EGF, VPA, and TGF β i) were needed to induce proliferation in the extrastriola, suggesting spatially distinct mitogenic response. While CHIR alone was insufficient to induce proliferation, there were many Edu+ SCs when CHIR was co-administered with EGF, VPA and TGF β i. Replacing CHIR with Wnt agonist or beta-catenin overexpression did not induce proliferation. Finally, β -catenin deletion did not prevent proliferation induced by 4F (CHIR EGF, VPA and TGF β i).

Conclusions: CHIR is necessary but insufficient to induce supporting cell proliferation in the neonatal mouse utricle. The mitogenic response differs between striolar and extrastriolar supporting cells: the former responds to CHIR and TGF β i, whereas the latter require the combination of CHIR, TGF β i, EGF, and VPA. Finally, CHIR induced proliferation in a Wnt/ β -catenin signaling independent manner.

SA204. Pharmacological Inhibition of TGF-B Signaling Impairs Supporting Cell Proliferation in the Injured Avian Basilar Papilla

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Background: Irreversible hearing loss in mammals stems from an inability to regenerate sensory hair cells. In contrast, birds robustly regenerate hair cells through the proliferation and subsequent differentiation of supporting cells, providing a model to discover pro-regenerative pathways. Our prior work identified a key signaling pathway that regulates supporting cell S-phase entry after hair cell injury. To further map the broader regulatory network, we mined our previously generated single-cell RNA-sequencing (scRNA-seq) datasets of the chicken basilar papilla (BP) across damage and regeneration time courses. This analysis identified TGF- β signaling as a candidate pathway with dynamic, time-ordered expression.

Methods: We analyzed scRNA-seq data from multiple post-injury time points to characterize expression changes in TGF- β pathway components and downstream targets. For functional validation in vivo, we performed lateral semicircular canal injections in post-hatch day 7 (P7) chickens. We co-administered the ototoxic antibiotic sisomicin with the pharmacological TGF- β inhibitor ITD-1 to suppress pathway activation in the damaged sensory epithelium. Supporting cell proliferation was assessed 48 hours post-injection by quantifying EdU incorporation, comparing ITD-1-treated BPs to vehicle controls subjected to identical ototoxic injury.

Results: Transcriptomic analysis revealed time-ordered modulation of TGF- β pathway genes during the transition from hair cell loss to the onset of regeneration. In situ, we observed a marked increase in expression of the ligand TGFB2 mRNA, coinciding with the onset of supporting cell proliferation. Pharmacological inhibition of TGF- β signaling in vivo significantly reduced EdU incorporation in supporting cells after ototoxic injury compared to controls.

Conclusions: Our findings implicate TGF- β signaling as a positive regulator of supporting cell proliferation in the avian auditory epithelium. The temporally distinct expression of TGF- β pathway components suggests a finely tuned regulatory role. Together with our prior work, these results support a model where precise timing and cooperation between TGF- β and other signaling pathways orchestrate cell cycle re-entry. Defining the temporal sequence and combinatorial interactions of these pathways is essential to elucidate the mechanisms of hair cell regeneration and to inform future therapies for human hearing loss.

Special Session 1: Restoring the Sixth Sense Through Vestibular Implantation - Clinical Trial Outcomes, Patient Perspectives and New Research Horizons

2:30 p.m. - 4:30 p.m.

Meeting Room 208ABC, Floor 2

Restoring the Sixth Sense Through Vestibular Implantation – Clinical Trial Outcomes, Patient Perspectives and New Research Horizons

Chair: Charles Della Santina, *Johns Hopkins School of Medicine*

Co-Chairs: Celia Fernandez Brillet, *Johns Hopkins University*; Angelica Perez Fornos, *Geneva University Hospitals*

First-In-Human Trial of the Labyrinth Devices Multichannel Vestibular Implant - Interim Results After 1-9 Years of Continuous Daily Use

Celia Fernandez Brillet¹, Margaret R Chow¹, Andrianna I. Ayiotis¹, Evan O. Vesper¹, Katherine N Mueller¹, Desi P. Schoo¹, Stephen Bowditch¹, Kelly E. Lane¹, Claudia N Lee¹, Lauren Trainer¹, Peter J. Boutros¹, Brian J. Morris¹, John P. Carey¹, Bryan K. Ward¹, Zubair Khan¹, Charles Della Santina*¹

¹*Johns Hopkins School of Medicine*

Individual Abstract: Purpose: Describe interim results for the first-in-human clinical trial of long-term, continuously motion-modulated prosthetic stimulation using the MVI™ Multichannel Vestibular Implant System developed by Labyrinth Devices LLC and MED-EL GmbH.

Methods: Twenty adults with bilateral vestibular hypofunction (11 ototoxic; 9 idiopathic/non-ototoxic/non-central) underwent unilateral implantation from August 2016 to May 2025. Duration from symptom onset to implantation ranged from 2-24 years. We assessed post-activation binocular 3-dimensional vestibulo-ocular reflex (3D VOR) responses to prosthetic stimulation and pre- to post-implantation changes in VOR responses to yaw, LARP and RALP head-on-body impulses and 0.1-2 Hz whole-body yaw rotation; pure tone and speech audiometry; dynamic visual acuity; clinical tests of posture and gait; and validated patient-reported outcome instruments for disability (Dizziness Handicap Inventory, Vestibular Activities of Daily Living) and health-related quality of life (SF36 and Health Utilities Index [HUI3]).

Results: Every implant recipient has used the system daily since device activation. Seven wear it 24 hr/day; others take it off while in bed. All but one have electrically-evoked VOR responses (typically ranging from ~5-100°/s and aligned approximately with the stimulated canal) for at least one electrode for each implanted canal. Rotary chair VOR responses are greater with motion-modulated stimulation than without. Motion perception thresholds are below VOR thresholds. Hearing sufficient for unaided telephone use was preserved in 11 of 19 implanted ears. Posture, gait, dizziness handicap, vestibular disability and health-related quality of life (quantified by SF36-derived SF6D utility) improved significantly compared to preop for the group. HUI3, which more heavily weights hearing, did not significantly change.

Conclusions: Vestibular implantation can be performed as an outpatient surgery while preserving useful hearing. Motion-modulated input generates motion percepts and 3DVOR responses consistent with semi-selective stimulation of each implanted canal, improving postural stability, gait, patient-reported disability and quality of life.

Support: NIDCD U01DC019364, R01DC13536; NIA R01AG076701; Labyrinth Devices LLC; MED-EL GmbH

Losing and Regaining Balance – Perspectives From the First Person to Undergo Vestibular Implantation and Long-Term Continuous Motion-Modulated Stimulation

Rick Stephens¹

¹*none*

Rick Stephens, *none*

Individual Abstract: Mr. Stephens will relate his experiences as both an individual who suddenly lost vestibular function due to gentamicin ototoxicity and the first person to undergo vestibular implantation and continuous, long-term motion-modulated stimulation.

Constant “Baseline” Electrical Stimulation Can Improve Function in Patients Implanted With Vestibulo-Cochlear Implants

Gautier Grouvel¹, Julie Corre¹, Jean-Francois Cugnot¹, Samuel Cavuscens², Maurizio Ranieri², Anissa Boutabla², Raymond van de Berg³, Stephanie Armand², Nils Guinand¹, Angelica Perez Fornos*¹

¹*Geneva University Hospitals*, ²*University of Geneva*, ³*Maastricht University Medical Center+*

Individual Abstract: Prototypes of vestibular implants are under development to rehabilitate patients with bilateral vestibulopathy (BV), mimicking the physiology of the vestibular system. An alternative and simplified approach consists in delivering only constant electrical stimulation to restore a “baseline” activity in the vestibular system. This study aimed to quantify the benefits of such a “baseline” stimulation (BS) delivered to semicircular canal afferents during prolonged periods of activation.

Two female patients with BV received a modified cochlear implant providing 3 extra-cochlear electrodes implanted in the semicircular canals. The patients’ speech processor was programmed to deliver a BS to the posterior semicircular canal for a period of 2.5 months. Objective and subjective measurements (gait analyses, questionnaires) were acquired throughout the stimulation period and afterwards over 6 sessions. DHI scores and number of steps for the tandem walk task were also analysed.

For patient-1, DHI scores improved from 52 to 30 throughout the BS, reflecting clinically relevant changes. After BS deactivation, DHI score increased to 48, representing a decline of functional status. The number of steps in the tandem walk task increased from 2 to 10 steps. Results for patient-2 do not present any changes during the BS.

Results in at least one patient demonstrate that vestibular deficits were reduced during the BS, complemented by positive patient feedback: “the implant allows me to walk in a straight line”. Objective and subjective gains disappeared when BS was deactivated. While this stimulation shows promise, its effectiveness may vary among patients, highlighting the need for devices that employ stimulation methods closer to human physiology.

Cognitive Assessment in Human Patients Equipped With a Vestibulo-Cochlear Implant

Anissa Boutabla*¹, Divya A. Chari², Gautier Grouvel³, Julie Corre³, Jean-Francois Cugnot³, Samuel Cavuscens¹, Maurizio Ranieri¹, Lukasz Bola¹, Raymond van de Berg⁴, Richard F. Lewis MD⁵, Nils Guinand³, Angelica Perez Fornos³

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Individual Abstract: Vestibular information improves navigation in rodents, but studies in human subjects with vestibular damage have been less conclusive. Herein, we investigated

the effects of short-term prosthetic vestibulo-cochlear implant (VCI) stimulation on navigation and neuropsychological function in patients with bilateral vestibulopathy. Specifically, two patients with severe bilateral vestibulopathy who underwent VCI surgery in 2021 completed a battery of neuropsychological tests, the Corsi Block Test (CBT), Digit Span Test (DST), and an egocentric mental transformation task (EMT), as well as a virtual reality-based spatial navigation task. This navigation task required subjects to perform a “return-to-home” challenge modeled on the triangle completion test under two distinct conditions: a dynamic condition involving yaw rotation and stepping in place, and a static condition in which navigation was performed via controller buttons while the head and body remained stationary. Testing was conducted at three time points: at least one month before stimulation, during active VCI stimulation, and at least one month after stimulation had ceased. During stimulation, both subjects demonstrated improved performance on the DST and EMT, while one also improved on the CBT. Furthermore, both subjects showed enhanced accuracy (angular error) and precision (standard deviation of angular error) in both dynamic and static conditions during VCI stimulation, with a subsequent decline in performance once stimulation was turned off. These preliminary findings suggest that VCI stimulation may positively influence higher-level cognitive and navigational functions in patients with bilateral vestibulopathy.

Optimization of Stimulation Parameters for a Vestibular Prosthesis: From Meniere’s Pacing to Bilateral Modulated Vestibular/Cochlear Stimulation

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Individual Abstract: Introduction: Vestibular loss is highly debilitating. Limited options exist for restorative therapy. One therapeutic approach is to provide an implantable neuroprosthesis. This presentation will discuss the development of a vestibular/cochlear neuroprosthesis by the group at the University of Washington, with emphasis on the optimization of stimulation parameters.

Methods: Non-human primates and human subjects were implanted with one or two unilateral vestibular or combined vestibular/cochlear prostheses. These devices were controlled and powered via an IR link to an external processor which could command stimulation based on sound and rotational inputs. Stimulation parameters could be preprogrammed or controlled via an attached computer. Stimulation was performed in the dark, during en-bloc rotation, and/or while tracking point targets or while undergoing optokinetic stimulation. Eye movements were recorded with video eye tracking in humans and implanted scleral coils in non-human primates. Neural responses in animals were recorded with tungsten microelectrodes. We used charge balanced biphasic pulse stimuli that could be modulated in pulse rate or current amplitude. Since there were multiple stimulation sites on each of 3 electrode arrays implanted adjacent to the ampulla of each semicircular canal, and remote ball and case grounds, monopolar and multipolar stimulation could be performed at each ampulla, or across ampullae. In addition, in animals that were implanted bilaterally, simultaneous stimulation could be performed at each ear, with different modulation phase relationships between ears.

Results: Each stimulation approach had strengths and weaknesses. The most natural stimulation was produced by bilateral prosthetic stimulation. Pulse rate modulation presented challenges at low stimulation frequencies. Current amplitude modulation showed current spread at higher current amplitudes. Interaural phase modulation produced consistent eye velocity changes for both pulse rate and current amplitude modulation strategies. Modulation produced changes in both velocity and direction of eye movements. Vestibular nucleus neurons and vestibular afferent fibers showed complex relationships between pulse amplitude and rate and the resulting neural discharge. Multipolar stimulation reduced current spread but also significantly reduced the efficacy of stimulation in eliciting eye velocity. Cross canal stimulation produced enhanced responses at some electrode sites, with the directional response being dominated by the most effective electrode. Implementation of complex transfer functions between the stimulus and the output of the implanted stimulator only partially compensated for the inherent limitations of the device.

Conclusion: Vestibular neuroprostheses have significant limitations which are only partially overcome by novel stimulation strategies.

Probing Central Vestibular Processing of Prosthetic Input via High-Density Neural Recordings in Freely Moving Nonhuman Primates

Ruihan Wei^{*1}, Olivia M. E. Leavitt², Oliver Stanley², Kantapon P. Wiboonsaksakul², Bassil Ramadan², Akshit Chhabra², Charles C Della Santina², Kathleen E. Cullen²

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Individual Abstract: Vestibular implants have shown promise in restoring function following bilateral vestibular loss (BVL), yet the central mechanisms by which artificial input is interpreted and integrated across sensorimotor pathways remain largely unexplored. To address this gap, we combine multichannel vestibular prosthesis (MVP) stimulation with high-density, single-unit population recordings in freely moving rhesus macaques. This platform enables us to investigate, with unprecedented resolution, how central vestibular and cerebellar circuits respond to prosthetic input across a broad repertoire of natural behaviors.

Monkeys with BVL were chronically implanted with MVPs targeting semicircular canal afferents. During experiments, animals engaged in naturalistic behaviors spanning multiple functional systems—including locomotion, voluntary gaze shifts, and postural stabilization—while receiving stimulation modulated in real time using inertial head velocity signals. To directly assess central processing, we used NHP high-density neural probes adapted for nonhuman primates to record from hundreds of simultaneously isolated units in vestibular nuclei and early cerebellar regions.

We found that MVP stimulation reduced head movement variability, stabilized gaze during locomotion, and partially restored short-latency postural responses to perturbations. Population-level recordings in the vestibular nuclei during naturalistic behaviors further revealed diverse single-neuron responses to prosthetic input. Standard stimulation protocols may induce maladaptive central adaptation, limiting long-term efficacy. Testing using novel waveform strategies—such as asymmetric pulses—evoked increased firing rates and reduced timing variability in some neurons, while others showed minimal modulation or phase-locked responses. Ongoing work is focused on how vestibular, cerebellar, and cortical circuits adapt over time to such prosthetic input, to inform stimulation strategies that avoid maladaptive plasticity and promote functional multisensory integration during self-motion.

This work establishes a unique translational platform to evaluate how the brain interprets artificial vestibular input in ecologically valid contexts. By linking neural activity across populations to behavior, we can identify key bottlenecks in central processing that may limit current prosthetic efficacy. Taken together, our findings underscore the need to refine stimulation strategies not only for reflexive responses but also for higher-order integration. Gaining a population-level understanding of how neural ensembles throughout the vestibular pathway—including cerebellar, thalamocortical, and cortical networks—encode prosthetic input will be essential for advancing stimulation design and improving clinical outcomes.

Prosthetic Stimulation of the Utricle and Saccule

Celia Fernandez Brillet^{*1}, Margaret R Chow², Kristin N Hageman², Gene Y Fridman², Charles C Della Santina²

¹*Johns Hopkins University*, ²*Johns Hopkins School of Medicine*

Individual Abstract: To date, electrical stimulation of the vestibular nerve has been mostly limited to targeting the three semicircular canals, which encode head angular velocity and acceleration. Stimulation of the two remaining vestibular end organs, the utricle and saccule, has been relatively unexplored because it is challenging to selectively target the densely packed, non-uniform, direction-encoding hair cells and nerve branches found in the otolith maculae. Our group characterized the normal otolith-ocular reflex (OOR) in chinchillas and showed the possibility of eliciting an OOR response using electrical stimulation, both in otherwise healthy and in diseased animals.

The microelectrode array (MEA) we used for that research comprised three shanks, each targeting specific regions: posterior canal, horizontal canal and saccule, and superior canal and utricle. Electrodes were inserted toward the maculae through ampullotomies on the horizontal and superior semicircular canals, precluding direct view of the targeted neuroepithelia. Although this method offered less visibility for electrode placement, it was chosen as a less-invasive approach with the goal of increasing the likelihood of hearing preservation. However, the planarity, stiffness and small dimensions of the array made insertion into the intended locations challenging and low yield.

Considering these challenges, we prioritized directly viewing the medial wall of the vestibule for precise and reliable identification of the utricle and saccule through a more invasive approach. We developed a new, custom-designed MEA based on skull prosections and a microMRI model of a chinchilla labyrinth and nerve anatomy. We designed the array to unfold and conform to the medial walls of the vestibule, fitting the anatomy and promoting consistent and successful MEA placement. It includes 45 pads targeting the utricle and saccule, plus two pads per semicircular canal. With this novel device, we intend to selectively and reliably stimulate the non-uniform branches innervating the otolith end organs.

Support:

The MEA described in this report was provided by the Polymer Implantable Electrode Foundry at the University of Southern California, which is funded under BRAIN Award U24NS113647. The project that gave rise to these results received the support of a fellowship from “la Caixa” Foundation (ID 100010434, fellowship code LCF/BQ/EU21/11890107) and Rafael del Pino Foundation.

Direct Current Stimulation of the Vestibular System Using Ionic Conduits in a Nonhuman Primate

Evan O. Vesper*¹, Katherine N. Mueller¹, Celia Fernandez Brillet¹, W. Mitchel Thomas¹, Brian J. Morris¹, Woo Seok Kang¹, Charles C. Della Santina¹, Gene Y. Fridman¹

¹*Johns Hopkins School of Medicine*

Individual Abstract: Modern neural implants use pulsatile, charge-balanced, biphasic stimulation to deliver information to nervous tissue. These electric pulses have a phase-locked excitatory effect on neurons that can be modulated by altering amplitude, frequency, phase duration, and interphase gap. While this approach avoids the generation of toxic byproducts at the electrode-tissue interface, pulsatile stimulation generally cannot inhibit neural activity—imposing limitations on its therapeutic effects. Ionic direct current stimulation (iDC) is a technique that can directly excite, inhibit, and sensitize neural tissue. This type of stimulation is delivered via ionic fluid conduits and can be paired with separated interface neural electrode systems to perform chronic experiments. Prior in-vivo rodent studies using iDC have noted its ability to suppress nociceptive fibers in peripheral nerves and broaden the dynamic range of the vestibulo-ocular reflex. However, long-term studies in larger animal models have not yet been conducted. In this paper, we present the first chronic implantation of iDC fluid conduits in the vestibular system of a nonhuman primate. We demonstrate the system's ability to deliver both conventional pulsatile and iDC stimulation and report on the implant's performance and stability over a six-month period.

The Future of Vestibular Implantation: Challenges, Opportunities and Controversies

Richard Lewis*¹

¹*Massachusetts Eye and Ear Infirmary, Harvard Medical School*

Individual Abstract: With Dr. Della Santina, Dr. Lewis will co-moderate a panel comprising all speakers in the session, with the group discussing open-ended questions about the future of vestibular implantation designed to elicit diverse opinions, spark convivial debate, and engage the audience.

Podium 1: Genetics of Hearing Loss: Screening, Sequencing and Outcomes

2:30 p.m. - 4:30 p.m.

Meeting Room 209ABC, Floor 2

Moderators: Paul Krumpoeck and Hela Azaiez

Preliminary Results From a Multicenter Evaluation of Hispanic Pediatric Genetic Hearing Loss

2:30 p.m. - 2:45 p.m.

Monica Rodriguez-Valero*¹, Shelby Redfield², Leonardo Elias Ordoñez Ordoñez³, Francisco Gonzalez Eslait⁴, Melania Abreu⁵, Alma Medrano¹, Alexandra Loosli⁶, Maria Paula Niño⁷, Juliana Vanessa Rincon⁸, Ana María Salamanca Guerrero¹⁰, Juanita Maria Mosquera

Huertas⁹, Jose Francisco Gallardo Ollervides¹⁰, Juan Carlos Cisneros Lesser¹⁰, Maria Fernanda Hinojosa Valencia¹¹, Paula Rivera Hoyos¹¹, Guadalupe Nogues Vizcaino¹², Laura Alonso Lujan¹², Diego Cuadros¹³, Julian Ramirez Cheyne¹³, Maira Alejandra Benavidez¹³, Mauricio Hernández-Carrillo¹³, Jorge Aliro Holguin Ruiz¹³, Eliot Shearer⁶

¹*Centro Medico ABC / Escuchar Sin Fronteras*, ²*Boston Children's Hospital*, ³*Clinica Universitaria Colombia, Fundacion Universitaria Sanitas, Hospital Militar Central, Universidad Militar Nueva Granada*, ⁴*Instituto para Niños Ciegos y Sordos del Valle del Cauca, Clinica Auditiva y Visual, Universidad del Valle Cali*, ⁵*Centro Médico ABC / Genos Medica / Escuchar sin Fronteras*, ⁶*Boston Children's Hospital, Harvard Medical School*, ⁷*Fundacion Universitaria Sanitas*, ⁸*Unidad de Investigación, Fundacion Sanitas*, ⁹*Universidad del Rosario*, ¹⁰*Centro Medico ABC / Escuchar Sin Fronteras / Hospital Angeles del Pedregal*, ¹¹*Escuchar Sin Fronteras / Aurea Lab*, ¹²*Escuchar Sin Fronteras*, ¹³*Hospital Universitario del Valle Evaristo Garcia*

Background: Pediatric hearing loss is a condition with diverse presentations and etiologies. Genetic evaluation is now considered the standard of care in this population. However, access to comprehensive genetic testing remains limited across Latin America for multiple reasons. The Latin American population has a wide range of genetic backgrounds, representing a unique ethnic group that, until now, has not been widely studied to determine genetic causes of hearing loss. Recent data show that Hispanic and Latino children with hearing loss have lower genetic testing diagnostic yields and significantly higher nondiagnostic or inconclusive results, despite similar prevalence rates of sensorineural hearing loss (SNHL) to their Asian and White counterparts. Few studies have evaluated genetic hearing loss in Latino populations using modern genomic technologies. In this study, we aimed to describe the results of genetic evaluations using comprehensive genomic testing in Hispanic children from Colombia, Mexico and the United States.

Methods: This multicenter study included hospitals in Colombia (Bogota and Cali), Mexico (Mexico City) and the United States (Boston). Data was collected from Hispanic children living in Colombia, Mexico and USA with SNHL who underwent hearing loss panels, commercial exome sequencing (ES) or custom targeted genomic enrichment and massively parallel sequencing (TGE+MPS). Variant pathogenicity was classified American College of Medical Genetics criteria.

Results: A total of 212 children (110 males) were evaluated; 66% were mestizos and 10% were white or white/caucasian. Congenital onset was present in 60% of cases and postlingual in 26% of the cases. Most patients (75%) underwent whole exome sequencing. Positive results were obtained in 111 probands (52%) and 51% of these cases were syndromic or syndromic mimic nonsyndromic hearing loss. Among probands with genetic etiology, 70 (66%) had an autosomal recessive inheritance. Bilateral hearing loss was present in 176 children; in this group, a genetic etiology was identified in 52%. Mutations in GJB2 accounted for 19 probands (20%), followed by, CDH7 (5%) SCL26A4 (5%), CDH23 (4%) and STRC (4%). Seventeen probands had unilateral hearing loss, with genetic etiology identified in only four cases (23%).

Conclusions: To our knowledge, this is the largest study to date describing the genetic etiology of hearing loss in Mexican, Colombian and Hispanic children. GJB2 mutations are the most common genetic cause of hearing loss in this population and syndromic diagnoses were higher than reported in other populations. Genetic evaluation in pediatric hearing loss is essential to personalize treatment and enable a multidisciplinary approach.

Continuous Updates to the Genomic Landscape of Deafness Associated Genes

2:45 p.m. - 3:00 p.m.

Brian Hong^{*1}, Hela Azaiez², Kevin T Booth², Terry Braun¹, Thomas Casavant¹, Genevieve Cox¹, Rose Gogal¹, Diana L. Kolbe³, Nathan Schaefer¹, Michael Schnieders¹, Richard J.H. Smith⁴

¹The University of Iowa, ²Indiana School of Medicine, ³University of Iowa Hospitals and Clinics, Molecular Otolaryngology and Renal Laboratories, ⁴Molecular Otolaryngology and Renal Research Labs, University of Iowa Carver College of Medicine

Background: Driven by the ever-increasing volume of exome and genome data, the classification of genetic variants remains a major challenge in providing precision medical care. The Deafness Variation Database (DVD) was developed to address this challenge in the domain of hearing loss by collating genetic, genomic, and phenotypic data with expert curation to provide Pathogenic/Likely Pathogenic/VUS/Likely Benign/Benign classifications for over millions of variants across 224 hearing-loss-associated genes.

Methods: The DVD v9.2 includes 224 genes known to cause hearing-loss related phenotypes. Based on human reference genome GRCh38 coordinates and uses GATK v4.2.6.1 Picard Liftover tool to include the GRCh37 coordinates, an internal computational pipeline, Kafeen, automates the classification of variants. The pipeline uses BCFtools to integrate information from gnomAD v4.1, ClinVar(2025-03-18 release), HGMD(2024.1 release) dbSNP(GCF_000001405.40). It implements VEP v111 plugins of computational tools: PhyloP, SIFT, LRT, MutationTaster, PolyPhen HDIV, GERP, REVEL v1.3, and CADD v1.7 to make predictions on the likelihood of variant pathogenicity. The front end web app provides a user friendly interface for the database and developed using the PHP Laravel framework.

Results: The DVD uses ACMG v3 guidelines to classify variants in 224 genes implicated in genetic hearing loss, using ClinVar and HGMD as published references and extracting minor allele frequencies for large scale populations from gnomAD v4.1. Computational tools assess conservation and deleteriousness to generate a composite pathogenicity score. The update to GRCh38 genomic coordinates allows for an increase in variant discovery. In aggregate, approximately 383,000 variants are classified as benign or likely benign, while 36,000 variants are classified as pathogenic or likely pathogenic.

Conclusions: The DVD provides a valuable resource for variant interpretation in the domain of genetic hearing loss. Ongoing efforts are focused on improving pathogenicity classification by integrating predictions of protein folding free energy differences with traditional sequence conservation based variant classifiers (e.g., CADD and REVEL) using a hearing-loss specific Bayesian framework. ACMG v4 guidelines will also be incorporated into the DVD.

An Ultra-Rapid Targeted Sequencing and Automated Bioinformatics Analysis for Genomic Newborn Hearing Screening to Reduce Time-To-Diagnosis for Hereditary Sensorineural Hearing Loss

3:00 p.m. - 3:15 p.m.

Shelby Redfield*¹, Charlotte Suh¹, Sierra Rios¹, Courtney French¹, Wanqing Shao¹, Cliff Li¹, Alexandra Loosli¹, Shira Rockowitz¹, Eliot Shearer²

¹*Boston Children's Hospital*, ²*Boston Children's Hospital, Harvard Medical School*

Background: The almost universal adoption of the newborn hearing screen (NBHS) in the United States has been instrumental in the early identification of infants with hearing loss. However, the NBHS is limited in which forms of sensorineural hearing loss it can detect, and it cannot identify genetic etiologies of hearing loss, which have important management implications. Prior studies have shown that the addition of limited genetic screening to the standard, physiologic NBHS improves screening sensitivity and decreases time to hearing habilitation. The purpose of this study is to develop a rapid, comprehensive, genomic NBHS that decreases time to diagnosis for children with hearing loss.

Methods: We obtained buccal swabs from newborns undergoing physiologic NBHS as standard-of-care. We performed rapid targeted genomic enrichment (Twist Biosciences) of definitive hearing loss genes (ClinGen Hearing Loss Gene Curation Expert Panel) followed by Illumina sequencing. To analyze the data, we developed an automated workflow integrating a standard bioinformatics analysis pipeline (Dragen, Illumina Inc) and a custom variant filtering process. The results of the genomic hearing screen (POSITIVE/NEGATIVE/INCONCLUSIVE) were provided to investigators in an emailed report. Positive results were reported when known or putative (likely) pathogenic variants that constitute a molecular diagnosis of nonsyndromic or syndromic hearing loss were identified. Variants of uncertain significance with congruous zygosity were called as inconclusive, as were heterozygous pathogenic variants in genes for which both autosomal dominant and recessive inheritance of hearing loss are possible. Secondary and incidental findings were excluded from analysis. The entire pipeline is automated with no human intervention required after sequencing is completed. Candidate variants were clinically confirmed prior to return of results with counseling.

Results: We have performed ultra-rapid comprehensive genomic NBHS for 50 controls and 69 subjects (119 in total). The total active time from sample collection to result was under 48 hours, with an average of 1 hour and 45 minutes for bioinformatic and variant analysis. There was complete concordance for 50 controls (28 gene-positive and 22 gene-negative) compared to standard-of-care genetic testing findings. Of the 69 subjects tested, 49 (71.0%) screened negative, 20 screened inconclusive (29.0%) and 1 screened positive (1.4%). The subject with a positive finding was diagnosed with mild-moderate sensorineural hearing loss on diagnostic auditory brainstem response testing. Clinical genetic confirmation testing of variants in OTOGL is pending. Inconclusive cases were reviewed by a genetic counselor and deemed nondiagnostic. Further enrollment of subjects and pipeline analysis is ongoing, including time-to-genetic-diagnosis comparison with a control group of newborns who did not undergo genomic NBHS.

Conclusions: Ultra-rapid genomic NBHS is a promising method to improve NBHS sensitivity and reduce time-to-diagnosis in newborns with hearing loss.

Comprehensive Whole Genome Sequencing Analysis of MYO15A and Genotype–Phenotype Correlations in Hearing Loss

3:15 p.m. - 3:30 p.m.

Yu Lu*¹, Jing Cheng¹

¹*West China Hospital, Sichuan University*

Background: In the Chinese population, MYO15A is the third most common deafness gene following GJB2 and SLC26A4. However, a substantial fraction of patients with hereditary hearing loss remain without a definitive molecular diagnosis, partly due to undetected pathogenic variants in MYO15A. These missed variants may reside in noncoding regions, poorly covered genomic segments, or represent structural variants (SVs). Addressing this diagnostic gap is essential for understanding the genetic architecture of MYO15A-related deafness.

Methods: We performed whole genome sequencing (WGS) on unsolved hearing loss cases, with a focus on individuals carrying single heterozygous pathogenic MYO15A variants identified in prior panel-based or exome testing. Comprehensive genomic interrogation included analysis of noncoding regions, low-coverage intervals, and structural variants. Candidate splice-altering and regulatory variants were validated through functional assays, including minigene splicing assays and transcript analysis. Genotype–phenotype correlations were explored with emphasis on recurrent mutational hotspots in the Chinese cohort.

Results: WGS significantly improved variant detection in MYO15A, especially in exon 2, which showed a markedly higher sensitivity compared to conventional methods. Fourteen distinct intronic variants were identified in 25 compound heterozygous hearing loss patients; splicing assays confirmed their disruption of canonical splicing, resulting in protein dysfunction. These discoveries increased the diagnostic yield for MYO15A-associated deafness by over 5%. Moreover, recurrent variants were enriched in exon 2, exon 64, and exon 65. Patients with mutations in these regions often presented with late-onset or moderate hearing loss, suggesting potential mechanistic differences compared to early-onset severe phenotypes.

Conclusions: This study demonstrates the power of whole genome sequencing in uncovering previously undetected pathogenic variants in MYO15A, including deep intronic and structural variants. By integrating functional validation, we establish the clinical relevance of these variants and refine genotype–phenotype correlations. Our findings highlight MYO15A as a major contributor to unsolved hereditary hearing loss in the Chinese population, expand its mutational spectrum, and suggest regional hotspots linked to variable clinical expressivity. Comprehensive WGS-based analysis thus represents an essential strategy to advance precision diagnostics and mechanistic understanding of MYO15A-related deafness.

Genetic Architecture of Tinnitus and its Relationship to Sensorineural Hearing Loss

3:30 p.m. - 3:45 p.m.

Caroline Nievergelt^{*1}, Adam X. Maihofer¹, Royce Clifford¹

¹*University of California - San Diego*

Background: Tinnitus is a highly prevalent multifactorial auditory disorder, whose pathophysiological mechanisms remain poorly known. Risk factors include hearing impairment, increasing age, and male sex, as well as environmental triggers such as noise exposure and stress. In addition, twin studies and recent large-scale genome-wide association studies (GWAS) have confirmed a heritable component to tinnitus. Further, tinnitus and sensorineural hearing loss (SNHL) are genetically highly correlated, with little indication of differentiating signals, and the divergent anatomic and genomic pathways for tinnitus from hearing damage remains unidentified. Here we dissect the genetic architecture of tinnitus from SNHL using the largest GWAS meta-analysis of tinnitus to date.

Methods: Genotype and phenotype data of over 750,000 individuals from the Million Veteran Program (MVP), the UK Biobanks (UKB), and the All of Us cohort were included. Tinnitus and SNHL were characterized by self-report and/or clinical diagnosis (ICD codes). GWAS was performed within each cohort in PLINK and inverse-variance weighted fixed effects meta-analysis was performed with METAL. Functional annotation was performed using FUMA, including MAGMA gene-based, pathway, and tissue expression analyses. Gene expression data of a human adult inner ear tissue (mostly from the vestibular system) was integrated to include disorder-relevant tissue for enrichment analyses. SNP based heritability and genetic correlation were estimated using LDSC. To estimate the genetic architecture of tinnitus and SNHL and the unique and shared polygenicity between the disorders, we performed univariate and bi-variate Gaussian mixer model analyses with MiXeR.

Results: A GWAS meta-analysis including 277,432 tinnitus cases and 475,916 controls identified 76 genome-wide significant loci, and gene-based analysis identified genes related to neuronal synapses and cochlear structural support. SNP-based heritability of tinnitus in individuals of European ancestry ranged from 6% (MVP, UKB) to 7% (All of Us). We found a large number of shared variants between tinnitus and SNHL, but also a distinct genetic architecture of tinnitus, with higher polygenicity and large proportion of variants not shared with hearing difficulty. Tissue expression analysis inferred broad enrichment across most brain tissues and the inner ear, in contrast to SNHL, which shows no enrichment in the brain, but enrichment in the inner ear, followed by nerve and pituitary tissues. Significant genetic correlations of tinnitus were found not only with SNHL, but also with psychiatric and health related traits.

Conclusions: We identified 76 tinnitus risk loci, almost doubling previous findings, and pointing to novel genes as well as known genes related to congenital and age-related hearing loss. Compared to SNHL, tinnitus has a higher polygenicity, and gene expression is enriched not only in the inner ear, but predominantly in the brain, establishing tinnitus as a distinct disorder separate from hearing loss. Finally, correlations of tinnitus with a spectrum of psychiatric disorders may provide potential new avenues for treatment.

Human Cochlear Cell-Specific Transcriptomes and Elevated Variants in Ménière's Disease and Hereditary Hearing Loss

3:45 p.m. - 4:00 p.m.

Samuel Adadey*¹, Shoujun Gu², Morag A Lewis³, Rafal Olszewski², Rabia Faridi⁴, Tyreek Jenkins⁵, Shelby Payne⁵, Kenechukwu Charles-Obi⁶, Robert J. Morell⁷, Thomas B. Friedman⁴, Bradley A. Schulte⁵, Karen P. Steel³, Judy R. Dubno⁵, Hainan Lang⁵, Michael Hoa²

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Background: The human cochlear transcriptome is crucial for identifying cell types and marker genes, and for understanding auditory function and inner ear disorders like Ménière's disease (MD) and hereditary hearing loss. MD is a debilitating chronic disease characterized by episodic vertigo, tinnitus, and fluctuating hearing loss, and is relatively understudied. We identified 34 distinct cell types in the human cochlea, uncovering its complex cellular diversity and novel marker genes.

Methods: We generated a comprehensive single-nucleus RNA-seq (snRNA-seq) reference transcriptome from human cochleae. Differential expression analyses combined with single-molecule fluorescence in situ hybridization were used to validate known markers and to identify new markers. To demonstrate the utility of our human snRNA-seq data, we mapped hereditary hearing loss genes across cochlear cell types and integrated variant data from the All of Us research program to identify genes associated with MD that show elevated variant loads in affected individuals. Leveraging genomic data involving 44,625 individuals from the All of Us Research Program, we conducted a case-control analysis to identify genetic variants significantly enriched in MD patients. Variant burden was assessed by comparing gene-level variant counts between MD and non-MD individuals.

Results: Based on our human snRNA-seq analysis, we annotated distinct cochlear cell types and identified novel cell type-specific marker genes. Cross-species comparison of these markers revealed strong conservation between humans and mice, supporting the continued use of mouse models in inner ear disease research. To explore the relevance of these findings to human hearing loss, we studied genetic variants linked to hereditary hearing loss and MD. Genes identified from this case-control analysis were then mapped onto our human cochlear snRNA-seq dataset to determine their expression across specific cochlear cell types. We identified candidate MD genes, including *BMPRI1B*, *FHOD3*, *LAMA2*, *RAB11FIP3*, and *SCN7A*, which showed cell type-specific expression within the cochlea, particularly in lateral wall structures. Expression of *Bmpr1b* in marginal cells was further validated in mouse cochlear tissue, highlighting the potential relevance of these genes to MD pathophysiology. Additionally, we used our transcriptomic resource to investigate the cellular expression patterns of genes implicated in hereditary hearing loss, providing a comprehensive framework for linking genetic risk to cochlear cell types.

Conclusions: Cross-species comparisons revealed strong conservation with mouse models, alongside key human-specific differences. Our dataset also enabled mapping of gene expression linked to Ménière's disease and hereditary hearing loss. This comprehensive human cochlear cellular transcriptome resource provides valuable translational insights, implicating specific cochlear cell types in the pathogenesis of MD and hereditary hearing loss.

Cochleovestibular Dysfunction in Patient With Primary Mitochondrial Disease Due to Pathogenic mtDNA Variants

4:00 p.m. - 4:15 p.m.

Johannes Ehinger*¹

¹*Lund University Hospital*

Background: Primary mitochondrial diseases (PMD) are a heterogeneous group of disorders, often with symptoms and findings from multiple organ systems, where pathogenic variants either in nuclear DNA (nDNA) or in mitochondrial DNA (mtDNA) affect mitochondrial function.

Methods: We have investigated how PMD affect hearing in one retrospective and one prospective cohort. Further, we have through video head impulse test (vHIT) and cervical vestibular evoked potentials (cVEMP) evaluated vestibular function in the prospective cohort of PMD patients.

Results: In the retrospective consecutive series of PMD patients seen at The Children's Hospital of Philadelphia, USA, SNHL was audiometrically confirmed in 20% of patients with pathogenic mtDNA variants, and in 58% of mtDNA deletions. In PMD due to pathogenic variants in mtDNA, onset of hearing loss was invariably post-lingual. In our ongoing prospective cohort of patients with PMD due to pathogenic mtDNA variants (primarily m.A3243A > G) at Skåne University Hospital, Sweden, to date 83% of subjects lack response to cVEMP on at least one side, while only 17 % had pathological vHIT.

Conclusions: That hearing loss due to mtDNA tend to have postlingual onset has important implications for the investigation of children and young adults with sensorineural hearing loss (SNHL). Genetic cause (mtDNA) of hearing loss has to be considered despite the child passing the newborn screen, and that whole genome sequencing also covering mtDNA should be considered the gold standard for investigation. SNHL is common in PMD, and vestibular dysfunction may be an underreported phenomena in this patient group, as imbalance and gait disorders in PMD is normally attributed to myopathy and fatigue. Here we show that vestibular dysfunction likely is an important contributor, and the inferior portion of the vestibular nerve seems to be affected earlier, as evident from the high proportion of pathogenic cVEMPs in this patient group.

FRMPD4 is Associated With X-Linked Non-Syndromic Hearing Loss

4:15 p.m. - 4:30 p.m.

Daniel Liedtke¹, Kristen Rak², Katrina M Schrode³, Philip Hehlert⁴, Niloofar Chamanrou⁵, Daniel Bengt¹, Radoslaw Katana⁴, Soganad Heydaran⁵, Julia Doll¹, Mei Han⁶, Indrajit Nanda¹, Pingkalai R Senthilan⁷, Linda Bieniussa², Johannes Voelker², Michaela AH Hofrichter¹, Jörg Schröder¹, Tobias Müller⁸, Marcus Dittrich¹, Oliver Bartsch⁹, Peter M Krawitz¹⁰, Eva Klopocki¹, Wafaa Shehata-Dieler², Reza Maroofian¹¹, Tao Wang⁶, Paul F Worley¹², Martin C Göpfert⁴, Hamid Galehdari⁵, Amanda M Lauer³, Thomas Haaf¹, Barbara Vona^{*13}

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Background: Understanding the full clinical spectrum of disease-associated genes is critical for advancing genomic medicine for accurate diagnosis and therapy. FRMPD4 (FERM and PDZ Domain Containing 4) has previously been linked to intellectual disability and epilepsy,

but its role in auditory function has not been explored. We investigated the potential contribution of FRMPD4 variants to non-syndromic sensorineural hearing loss (NSHL) and examined conserved molecular mechanisms across species.

Methods: Exome sequencing was performed in two unrelated families with maternally inherited missense variants in FRMPD4. Clinical phenotyping included longitudinal audiological assessments. To assess evolutionary conservation and functional mechanisms, we analyzed FRMPD4 expression in mouse, zebrafish, and *Drosophila* auditory tissues. Functional assays included mouse auditory brainstem response (ABR) testing, zebrafish knockdown and mutant models with phenotypic evaluation of otic vesicle and posterior lateral line development, neuromast morphology, and acoustic startle responses, as well as behavioral assays of sound perception in *Drosophila* mutants.

Results: Three affected males presented with prelingual, bilateral, symmetric hearing loss, including congenital onset in one individual. No neurodevelopmental features were observed, and over 20 years of clinical follow-up in one family excluded syndromic involvement. Cross-species analyses showed conserved FRMPD4 expression in auditory structures. Zebrafish loss-of-function models demonstrated reduced neuronal populations in the otic vesicle and posterior lateral line, impaired neuromast development, and diminished acoustic startle responses, indicating impaired auditory processing. In mice, ABR testing revealed high-frequency hearing loss that paralleled the human phenotype. *Drosophila* mutants displayed quantifiable deficits in sound perception, supporting a conserved molecular role across vertebrates and invertebrates.

Conclusions: This study identifies FRMPD4 variants as a novel cause of non-syndromic sensorineural hearing loss, expanding its functional repertoire beyond neurodevelopmental disorders. The convergence of human genetics and cross-species functional evidence highlights a conserved auditory role for FRMPD4 and suggests that disruption of neuronal and synaptic pathways underlies hearing impairment. These findings broaden the phenotypic spectrum of FRMPD4, carry direct implications for genetic diagnosis and counseling in hearing loss, and establish new avenues for mechanistic and therapeutic research in auditory biology.

Podium 2: Neural Mechanisms of Speech Perception and Understanding

2:30 p.m. - 4:30 p.m.

Ballroom B, Floor 3

Moderators: Ciaran Stone and Jessica MacLean

Electroencephalographic Measurements of Speech Detection in Noise in the First Year of Life

2:30 p.m. - 2:45 p.m.

Laurianne Cabrera*¹, Clémence Basire²

¹CNRS, ²Université de Paris - CNRS

Background: Infants are known to have more difficulties detecting speech in the presence of a noisy background compared to adults. Most of the previous studies used behavioral methods to assess this capacity, whereas electroencephalography (EEG) may provide objective neural markers of the development of this ability. Our study aims to assess infants'

speech detection abilities in a noisy background by examining the auditory event related potentials (ERP) using a passive listening procedure.

Methods: We recruited a group of 25 three-month-old infants with normal hearing and typical development, who were following-up at 10 months of age. We also recruited a group of 31 adults for comparisons. All listeners were awake throughout the EEG recordings and were presented with the syllables "ba" or "da" in a continuous stationary speech-shaped noise played at 65 dB SPL via two speakers in front of the listeners. The syllables were randomly played at four different signal-to-noise ratios (SNR = +3, -4, -8, -15 dB). A total of 52 trials per SNR was played while measuring ERPs for about 20 min. The ERP amplitude after syllable onset was averaged over five frontal electrodes and in the time window 400:600 ms, corresponding to the P300-like ERP response.

Results: A first analysis showed that for both infant groups and the adult group, only ERP amplitude obtained at the SNR -15 dB were not significantly different from the neural responses obtained during the noise inter-trial presentation (considered as the baseline response when no speech was presented). A second analysis using a linear-mixed model was run on the mean [400-600ms] ERP amplitude and revealed a significant effect of Age ($p = .008$), SNR level ($p < .001$) and interaction between these two factors ($p = .020$). Post-hoc analyses indicated that at 3 months of age, decreasing the SNR significantly decreased the ERP amplitude, while at 10 months of age the ERP amplitude differed significantly between the -15 and +3, -15 and -4, and -8 and +3 conditions, but not between any other pairs.

Conclusions: These findings revealed, at both 3 and 10 months of age, an effect of SNR on the P300-like amplitude related to syllable detection in noise: the greater SNRs, the greater the ERP amplitude. This result is in line with adult studies showing that the P300 amplitude decreases as the difficulty of discriminating stimuli increases. Importantly, at 3 and 10 months, ERP responses were significantly different from neural noise, starting at SNR equal to -8 dB. This finding suggests that as for the adults, the infants' brain is able to detect syllable at this SNR level by 3 months of age. Our results are promising for objective clinical measures of speech-in-noise development during infancy.

Speech-In-Noise as a Biomarker of Peripheral Auditory Neuropathy in Metabolic Disorders: Proof of Concept in Patients With Uncontrolled Type 2 Diabetes

2:45 p.m. - 3:00 p.m.

Jerome Nevoux^{*1}, Fabrice Giraudet², Camille Rochmann³, Kaier-Green Zoe³, Blandine Duval³, Laullier Hugo³, Laura Breda³, Mathieu Schué³

¹CHU Bicêtre, ²UMR INSERM 1107, University Clermont Auvergne, Institute of Hearing, University Hospital of Clermont-Ferrand, SoluSons, Clermont-Ferrand, ³CILcare

Background: Sensorineural hearing loss (SNHL) is an underrecognized comorbidity of type 2 diabetes (T2D).

Peripheral auditory neuropathies, including cochlear synaptopathy and cochlear microangiopathy, and chronic systemic inflammation affecting central auditory pathways are potential mechanisms.

These factors may contribute to early auditory dysfunction, specifically speech-in-noise (SIN) comprehension difficulties with standard hearing thresholds within normal limits, often reported as "hidden hearing loss".

Methods: DIAMANT was a French, multicenter, cross-sectional, prospective study conducted at three diabetology centers. It aimed to evaluate the prevalence of different auditory dysfunctions among an uncontrolled population with T2D, especially SNHL and identify effective tools to distinguish them.

Participants who consented (aged 25–70, T2D > 2 years, HbA1c > 7%) underwent a comprehensive battery of tests : standard (0.25-8 kHz) and extended high frequency (8–16 kHz) pure-tone audiometry, SIN testing with the French Matrix test (Framatrix), Distortion Product OtoAcoustic Emissions (DPOAE), electrocochleography (EcochG), and cognitive screening via the Montreal Cognitive Assessment (MoCA).

Participants with abnormal otoscopy, tympanometry, conductive / asymmetrical hearing loss were excluded.

Results: Among 241 participants (61% women, median age 60 [IQR:53.0-64.0]), three hearing profiles emerged:

- Normal hearing (NH, standard pure-tone average at 0.5,1, 2, 4 kHz (PTAv) < 25 dB and Framatrix < −3 dB): 45.6%

- SIN-Deficit (SIN-D, PTAv < 25 dB and Framatrix $\geq$ −3 dB): 37.4%

- Hearing loss (HL, PTAv $\geq$ 25 dB): 17%

After excluding those with cognitive impairment (MoCA < 26), 137 participants remained: 59.8% NH, 26.3% SIN-D, and 13.9% HL.

In NH and SIN-D, DPOAE were present (90.7%), or partially present (5.6%).

Audiometry revealed significant differences in hearing thresholds between NH and HL across all frequencies. NH and SIN-D had similar thresholds at low frequencies (0.25–1 kHz), but SIN-D diverged at mid-to-high frequencies ($\geq$ 2 kHz), no longer resembling HL at 8-16 kHz.

Framatrix scores were significantly correlated with PTAv low, PTAv mid, PTAv high, indicating SIN performance deteriorates as pure-tone sensitivity declines.

SIN testing effectively distinguished SIN-D and NH per the median 2 dB signal-to-noise ratio difference observed.

At the group level, EcochG revealed significantly reduced auditory nerve response in SIN-D compared to NH, reflecting a decrease or degeneration of synapses and/or spiral ganglion cells. At the individual level, EcochG correlated with Framatrix scores and PTAv at mid-to-high frequencies, but not at low frequencies. This reinforces the concept that EcochG and SIN testing can identify emerging deficits, specifically at mid-high frequencies.

Conclusions: Although SNHL prevalence in T2D was not higher than in the general population, SIN deficit with normal hearing was more frequent.

For SIN-D, outer hair cell dysfunction can be excluded and peripheral auditory neuropathy suspected when controlling for cognitive factors.

SIN testing offers a practical, non-invasive alternative to electrophysiology and should be integrated into routine auditory testing.

More work should confirm SIN testing as a biomarker of peripheral auditory neuropathy.

A Cortical Hierarchy of Temporal Integration Windows During Naturalistic Speech Processing in Children

3:00 p.m. - 3:15 p.m.

Tong Shan*¹, Dawlat El-said¹, Kelly Eggen¹, Mia Obermueller¹, Carlo de los Angeles¹, Montana Wilson¹, Joshua Medor¹, Warsan Ali¹, Daniel Abrams¹

¹*Stanford University*

Background: Real-life verbal communication depends on the brain's ability to dynamically integrate speech over time, allowing listeners to derive meaning. Recent neuroimaging methods enable investigation of comprehension during naturalistic communication. Prior studies in adults have demonstrated a hierarchy of temporal receptive windows, with auditory cortices processing short timescales and the default mode network (DMN) and lateral frontoparietal network (LFPN) supporting integration over longer timescales. However, the neurodevelopmental trajectories of these systems in children, and their relationship to narrative comprehension and social communication abilities, remain largely unknown. The present study investigates the developmental mechanisms underlying comprehension during naturalistic speech in typically developing children.

Methods: Twelve neurotypical children (ages 9–12 years; 3 female), all native English speakers, participated in an fMRI study. Scanning was performed on a 3T MRI scanner (48-channel coil, TR = 800 ms). During scanning, participants listened to (1) natural narratives read by a female speaker, (2) a word-scrambled version of the narrative, and (3) a sentence-scrambled version. Compositional and pragmatic comprehension of the narrative were assessed following each narrative. For each condition, inter-subject synchronization (ISS) analysis was performed. Voxel-wise correlation coefficients were computed across listeners to assess the reliability of BOLD response time courses to each stimulus. ISS differences between intact and scrambled narratives were computed.

Results: Neurotypical children showed high comprehension accuracy (> 90%) for both compositional and pragmatic content across narratives, indicating that the children heard and understood the narratives during fMRI data collection. Consistent with findings in adults (Lerner et al., 2011), robust ISS was observed in auditory cortex, including Heschl's Gyrus, Planum temporale and Superior Temporal Gyrus, across all conditions, indicating short-timescale integration in superior temporal regions. However, only the intact narrative elicited significant ISS in the precuneus and angular gyrus of the DMN, as well as in the superior parietal lobule and dorsolateral prefrontal cortex of the LFPN. In contrast, the word- and sentence-scrambled narratives failed to elicit ISS in DMN or LFPN regions, except for the precuneus in the sentence-scrambled condition. Results are consistent with the hypothesis that DMN and LFPN support long-timescale integration.

Conclusions: Preliminary findings suggest that, in children, DMN and LFPN support long-timescale integration of naturalistic narratives, whereas auditory cortices reliably encode shorter timescales associated with word-level processing. Findings suggest that a cortical hierarchy for temporal analysis of naturalistic speech is intact in school-aged children. Future analyses will examine relationships between individual differences in temporal integration of speech and neuropsychological measures of language and social communication abilities as well as brain network connectivity underlying temporal integration.

Central Auditory Function is Related to Earlier Reading Development in Childhood

3:15 p.m. - 3:30 p.m.

Malina Krotzer*¹, Christopher Niemczak², Shireen Geimer², Monika Adhikari², Suwen Wu², Samantha Leigh², Jonathan Lichtenstein³, Odile Clavier⁴, Albert Magohe⁵, Enica Massawe⁵, Jay Buckley²

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⁴Creare LLC, ⁵Muhimbili University of Health and Allied Sciences

Background: Central auditory tests assess the brain, which is crucial for reading development in children. Therefore, testing central auditory function in early childhood (< age 5) may provide a marker for reading ability in later childhood. This may be particularly useful in low- middle-income countries where testing resources are limited.

Methods: We studied 169 children from an ongoing longitudinal study in Tanzania that entered the study by age 5 and were followed for at least 4 visits (follow-up between 1.5 and 6 years). For all children, a polynomial curve was fit to triple digit test (TDT – speech perception in noise) performance across age to provide a normal age trajectory of the test in the cohort. High-performers were identified as those who consistently performed better than the age-appropriate mean value.

We assessed subsequent oral reading fluency in high-performers and the comparison group on the early grade reading assessment (EGRA).

We treated performance on the oral fluency task in 5 point increments (from 0 to 60, the maximum score) on the EGRA as discrete Performance Levels (PL). We treated “age at first attainment of PL” as a time-to-event outcome and constructed interval-censored event times from longitudinal scores on the reading assessment. We fit log-logistic accelerated failure time (AFT) models with group (high-performers vs. comparison) as the only predictor to estimate median ages for reaching each PL for our two groups.

Results: Plotting performance on the oral fluency task against age showed that on average, the high-performer group had better reading ability compared to same-age children in the comparison group from 5-11 years of age.

Across PLs 10–60, the estimated difference in median age to reach the threshold ranged from 0.95 to 1.72 years, exhibiting an increasing lag between groups at higher PLs. The corresponding time ratios comparing high-performers to the comparison group were consistently between 0.85 and 0.90, indicating that high-performers progressed in oral fluency approximately 10–15% earlier than the comparison group across the entire range of PLs.

Specifically, high-performers reached the PL of 50 (out of 60 words) at a median age of 9.4 years, whereas the comparison group reached it at 10.7 years, with an estimated time lag of 1.3 years (95% CI: 0.61, 2.04 years).

Conclusions: Using interval-censored AFT models, we found that children who are consistently high-performers on TDT in early years show a stable 10%-15% acceleration (earlier attainment) of oral fluency skills compared to those performing at average or below-average on the TDT for their age.

Elliptical Speech Reveals the Use of Broad Phonetic Categories Aids Noise-Degraded Speech Perception

3:30 p.m. - 3:45 p.m.

Gavin Bidelman*¹, Zara Eisenhut¹, Lucy Borowski¹, Rose Rizzi¹, David Pisoni¹

¹*Indiana University*

Background: Speech perception requires that listeners both classify sensory information into smaller groupings while also coping with noise that often corrupts the speech signal. The strength of categorization and speech-in-noise (SIN) abilities show stark individual differences. Some listeners perceive speech sounds in a gradient fashion, while others categorize in a discrete/binary manner, favoring fine acoustic details vs. a more abstract phonetic code, respectively. Prior work suggests SIN processing is (i) related to more gradient phonetic perception and (ii) varies with musical training.

Methods: To further probe relations between perceptual gradiency and noise-degraded listening, we measured phoneme categorization (identification labeling), SIN recognition (QuickSIN), and sentence recognition in listeners with varying musical background. Categorization was measured for vowels and stops using standard labeling tasks. Sentence-level speech recognition and discrimination were assessed using “elliptical speech” [Miller and Nicely. (1955). *J. Acoust. Soc. Am.*, 27, 338–352], which use featural substitutions that renders them meaningless under normal (clean) conditions but surprisingly improves their recognition under noise degradation. We hypothesized listeners who use broader perceptual equivalency classes in hearing elliptical speech would show better SIN perception, indicative of a more gradient listening strategy.

Results: Listeners perceived elliptical sentences as different than their intact counterparts in the clear (as expected) but, surprisingly, as sounding the same under noise degradation. However, this elliptical noise-related benefit varied with music background. Nonmusicians showed larger “susceptibility” to ellipses than musicians, consistent with the notion they used broader phonetic categories (i.e., more gradient listening) than their musician peers. Relatedly, more gradient listeners had larger elliptical noise benefit, indicative of broader category use. Elliptical speech perception was also associated with QuickSIN performance in both groups but in opposite ways. Use of broader categories was related to better SIN processing in nonmusicians but poorer SIN processing in musicians.

Conclusions: Collectively, our findings suggest listeners can use broader perceptual equivalence classes to deal with degraded listening situations but this depends critically on their prior auditory experience. Nonmusicians might use broader phonetic categories to assist SIN perception while musicians might use narrower categories in otherwise similar speech contexts.

Interpretable Embeddings of Speech Explain and Enhance Brain Encoding Performance of Audio Models

3:45 p.m. - 4:00 p.m.

Riki Shimizu*¹, Richard Antonello¹, Chandan Singh², Nima Mesgarani¹

¹*Columbia University*, ²*Microsoft Research*

Background: Self-supervised speech models (SSMs), such as HuBERT, are increasingly hailed as powerful computational models of human speech perception, as their embeddings are shown to be highly effective in predicting brain responses to speech. However, since their representations are inherently black-box, it remains unclear what drives their alignment with brain responses. Although representational similarity analysis or linear probing of their representations provides insight into what these models encode, they do not reveal which representational components are responsible for alignment with neural activity. To establish a stronger neuroscientific basis for the alignment between SSMs and neural responses for

speech perception, it is essential to directly test which features of SSM representations account for their predictive power.

Methods: In this study, we analyzed open-source electrocorticography (ECoG) recordings collected from nine neurosurgical patients as they listened to naturalistic speech stimuli. To investigate the representational basis of SSM-brain alignment, we constructed linear encoding models of neural responses using banded ridge regression. These models incorporated hand-crafted, interpretable features—spanning acoustic to lexical domains—and contextualized embeddings from three state-of-the-art speech models: Whisper, HuBERT, and WavLM. By applying a variance partitioning approach, we quantified the extent to which ECoG response variance was shared between two feature classes, directly testing the representational basis of SSM–brain alignment.

Results: We first demonstrate that the SSMs' alignment with the brain can be mostly explained by their ability to learn and encode simple interpretable speech features. Additionally, SSMs exhibit a systematic trade-off between encoding of brain-relevant low-level and high-level features across layers. Finally, our results show compellingly that SSMs learn brain-relevant semantics that cannot be explained by lower-level speech features, with this capacity increasing with model size and context length. Together, these findings suggest a principled approach to building better and more efficient encoding models of the brain by augmenting SSM embeddings with interpretable features.

Conclusions: By applying the variance-partitioning approach to a diverse set of interpretable features and SSM representations, we identify which representational features are responsible for SSM's ability to predict brain responses for speech perception. Our results further demonstrated that augmenting SSM embeddings with interpretable features yields more accurate and efficient encoding models, highlighting the value of refined interpretable feature sets. Importantly, although our interpretable features account for most of the neural variance captured by SSM representations, a statistically significant portion remains unexplained. Identifying the nature of this residual variance is an important direction for future research.

Linking EEG Evoked Potentials to Dimensions of Lexical Competition in Cochlear Implant Users

4:00 p.m. - 4:15 p.m.

Francis Smith^{*1}, Sarah Colby², Nour Alsabbagh¹, Joel Berger¹, Phillip Gander¹, Timothy Griffiths³, Bob McMurray¹, Inyong Choi¹

¹University of Iowa, ²University of Ottawa, ³Newcastle University

Background: One key challenge in spoken word recognition is the temporary ambiguity that results from speech unfolding over time. Models of speech perception which are based on research with normal hearing (NH) listeners resolve this ambiguity through incremental processing and competition among lexical candidates. Multiple studies have found that these patterns of competition differ in cochlear implant (CI) users (Farris-Trimble et al., 2014; McMurray et al., 2017) and recent work has revealed that this basic mechanism of competition varies along three principal dimensions across both CI users and NH listeners: delayed lexical access (“wait-and-see”), resolution of competition (“sustained-activation”), and overall rate of activation (McMurray et al., 2024). The neural substrates underlying these dimensions of lexical access and the sources of individual variability remain unclear. We examined a subset of CI participants from McMurray et al. (2024) to investigate the neural correlates of these three dimensions of word recognition in CI users.

Methods: We tested 63 experienced (> 1 year of device use) CI users (mean age = 60.5 years) from McMurray et al. (2024) who also participated in a speech recognition task (the Iowa Test of Consonant Perception: ITCP; Geller et al., 2020, 2021; Smith et al., 2025) while 64-channel EEG data were collected. The ITCP is a four-alternative forced choice speech perception task. Monosyllabic words were presented in multi-talker babble in both high (+15 dB) and low (+7.5 dB) signal-to-noise ratio trials. We used data from Cz to analyze early and late evoked activity to the onset of babble and the onset of the target word and correlated these measures with each of the three dimensions of word recognition described above. This yielded eight EEG measures (early/late $\times$ noise/target $\times$ amplitude/latency), which we correlated with competition dimensions.

Results: Participants who showed lower EEG amplitudes in response to the target word onset (both early and late peaks) were more likely to be more “wait-and-see” in their eye movement patterns ($r = -.22$, $r = -.20$). Participants who showed delayed latency in their late activity in response to the target were more likely to show “sustained activation” eye movement patterns ($r = .31$). Participants who showed delayed latency in their early activity in response to the initial noise were more likely to show “slow activation rate” eye movement patterns ($r = .29$).

Conclusions: Within this large cohort of CI users, we found significant correlations between EEG evoked potentials collected in a speech perception task and patterns of word recognition collected in a separate visual world paradigm task. There was substantial variability in both EEG measures and word recognition dimension measures among the CI population measured in this study, which matches the large variability in lexical competition profiles shown by this group.

Neural Encoding of Acoustic and Linguistic Speech Features Reveals Correlates of Understanding

4:15 p.m. - 4:30 p.m.

Alexis MacIntyre^{*1}, Tobias Goehring², Matt Davis³

¹*University of Cambridge*, ²*Cambridge Hearing Group, University of Cambridge*,

³*Cambridge Hearing Group, MRC Cognition and Brain Sciences Unit, University of Cambridge*

Background: How listeners parse a variable and continuous acoustic speech signal into meaningful, abstracted representations—language—is a question central to auditory neuroscience. Moreover, the resilience of this process to multiple forms of signal degradation is not fully understood. Theory suggests that listeners benefit from simultaneous and redundant cues that situationally differ in their availability and utility. These cues can range from higher-level information, such as semantic context, to low-level acoustic properties, such as spectral contrasts; however, previous studies investigating low-level speech cues tend to examine short, discrete stimuli in the context of behavioural paradigms. Hence, it is unclear how low-level spectral properties are neurally encoded and whether this can be detected using non-invasive electrophysiology during continuous speech listening.

Methods: The current study consists of a listening task wherein $n = 38$ young, typically hearing adults were presented with a naturalistic story whilst undergoing continuous electroencephalography (EEG). Critically, access to speech cues was manipulated over two dimensions: Acoustic spectral clarity and linguistic familiarity, with the latter contrast consisting of a familiar language paired against an unfamiliar but closely related language,

both produced by the same bilingual speaker. Using banded regression, we modelled neural responses to a set of spectro-temporal and linguistically derived features, including lexical surprisal scores obtained from large language models. To disentangle correlated predictors and ensure robustness of results, we subjected encoder weights to a random permutation analysis. We performed comparisons across listening conditions at the group level as well as a data-driven analysis of individual differences in self-reported understanding.

Results: The results reveal unique contributions from four acoustic properties: The amplitude envelope; peaks in the envelope first derivative; spectral variation, an intensity-invariant measure of spectral change; and spectral flux, which reflects both spectral and signal energy change. When introducing higher-level features to the acoustic encoding model, we found substantive word onset encoding that was specific to intelligible speech, but minimal additional variance explained by phoneme and syllable onsets or surprisal. We identified features, time windows, and channel clusters in which the neural response was mediated by language, acoustic clarity, or their interaction. Finally, differences in the neural response across listening conditions could be summarised as a composite score that strongly predicted subjective individual comprehension of the most spectrally degraded but moderately intelligible speech ($\text{Rho} = 0.90$, $p < .001$).

Conclusions: These findings underscore the complex interplay between low- and high-level features during naturalistic speech perception and illustrate inter-listener as well as inter-condition differences in the neural encoding of such features. Syllable and phoneme onset encoding may be driven by correlated acoustic properties; however, word onset encoding persists after controlling for low-level acoustics. The observed brain-behavioural link between feature encoding and intelligibility may hold promise for future interventions.

Honorary Symposium: Cortical Codes, Psychophysics and Prostheses at the Cocktail Party: The Contributions of John C. Middlebrooks

4:45 p.m. - 5:45 p.m.

Ballroom B, Floor 3

Chair: Julie Arenberg, *Massachusetts Eye and Ear Infirmary, Eaton Peabody Laboratory*

How Do Cortical Neurons Represent Auditory Space?

G. Christopher Stecker, *Boys Town National Research Hospital*

Spatial Selectivity and Stream Segregation in Humans, Animals, and Neurons

Justin Yao, *Rutgers University*

Complex Sound Localization by Human Listeners

Ewan Macpherson, *Western University*

Probing the Auditory Pathway With Auditory Prostheses

Julie Arenberg, *Massachusetts Eye and Ear Infirmary, Eaton Peabody Laboratory*

Electrophysiological and Behavioral Investigations of Electrical Stimulation in Cats and Humans

Matthew L. Richardson, *Center for Hearing Research, University of California, Irvine*

Electrophysiological and Behavioral Investigations of Electrical Stimulation in Cats and Humans

Robert Carlyon, *Cambridge Hearing Group, University of Cambridge*

Challenging the Status Quo -- Or Welcoming Serendipity

John Middlebrooks, *UC Irvine*

Poster Blitz Session I

4:45 p.m. - 5:45 p.m.

Meeting Room 208ABC, Floor 2

1. Hearing Loss and Vocalization Deficits in Two Mouse Models of Alzheimer's Disease

Sevda Abdavinejad^{*1}, Bridget Crandall¹, Emma Ogilvie¹, Amanda Lauer², Micheal Dent¹

¹*The University at Buffalo - SUNY*, ²*Johns Hopkins University*

Background: Alzheimer's disease (AD) is the most common cause of dementia, affecting millions of people worldwide. In addition to memory decline, AD is also often associated with early-onset hearing loss and vocal communication impairments. While hearing loss has been identified as the most modifiable risk factor for dementia, the mechanisms linking it to AD remain poorly understood. Transgenic mouse models carrying human genes connected to familial AD can be a powerful tool to examine these associations. APP/PS1 mice capture the deposition of extracellular amyloid plaque pathology seen in AD patients, while 3xTg-AD mice display both amyloid plaques and intracellular neurofibrillary tangles (NFTs). The present study investigated hearing thresholds and ultrasonic vocalizations (USVs), a measure of mouse social communication, across the lifespan in these widely used AD models. Hearing was assessed using behavioral and psychoacoustic techniques that engage attention, memory, and participation, providing greater sensitivity than physiological measures.

Methods: Behavioral hearing thresholds were collected daily in male and female 3xTg-AD, APP/PS1, and wild-type (WT) control mice (n = 5 per sex per strain) across the lifespan using operant conditioning and psychophysical techniques. The same mice were recorded for USV assessment at four time points (11, 24, 52, and 68 weeks of age) across three social conditions: isolation, same-sex, and opposite-sex pairs. Calls were analyzed for number, type, bandwidth, and duration. Analyses compared outcomes across age, strain, sex, and social condition.

Results: Behavioral thresholds revealed progressive, age-related hearing loss in both sexes across all strains, which was positively correlated with a reduced number and diversity of USVs. 3xTg-AD mice exhibited significantly earlier-onset hearing loss than both WT and APP/PS1 mice, suggesting a role for NFT-specific pathology or the combination of plaques and tangles in auditory and vocal communication declines. In contrast, APP/PS1 mice showed only age-related hearing loss comparable to their WT controls. USV analysis revealed age-related reductions in call number and repertoire diversity in both models, with 3xTg-AD mice showing the most severe impairments. A sex effect was observed in the

3xTg-AD model, with males exhibiting more severe hearing and vocalization decline than females.

Conclusions: Hearing loss was positively correlated with reduced vocalization output, indicating that auditory decline contributes directly to impaired social communication in AD models. Findings demonstrate that hearing and communication deficits are early, progressive features of familial AD mouse models. The 3xTg-AD line more closely recapitulates human patterns of hearing loss and vocal communication decline, underscoring the significant role of NFTs or combined plaque and tangle pathology in familial AD. These results support USVs and auditory thresholds as promising biomarkers for early AD-related cognitive impairment and highlight the need for further studies on the mechanistic links between AD pathology, auditory processing, and communication deficits.

2. From Sound to Movement: The Neural Backbone of the Acoustic Startle Reflex

Jan Frederik Ahrend*¹, Jana Erlmoser¹, Kathrin Deborah Wicke², Felix Felmy², Christian Vogl¹

¹*Auditory Neuroscience Group, Institute of Physiology, Medical University Innsbruck,*

²*Institute of Zoology, University of Veterinary Medicine Hannover Foundation,*

Background: The acoustic startle reflex (ASR) is a fast bodily reaction to a loud and unexpected auditory stimulus – a protective mechanism inherent to most mammalian species. On cellular level, large cochlear root neurons (CRNs), located between the auditory periphery and cochlear nucleus, have been identified as the first neural substrate of the underlying reflex circuitry. CRNs receive glutamatergic input from primary afferent spiral ganglion neuron (SGN) collaterals and were shown to be subject to various efferent modulatory pathways. Their large axons mainly target neurons of the contralateral pontine reticular nucleus (PnC) formation, which project onto cranial and spinal motor neurons and are in turn subject to extensive modulatory inputs from various brain areas. Despite their fundamental role in a survival reflex circuit, CRNs are still remarkably poorly characterized in regard to their cellular excitability and activity patterns as well as their molecular composition and morphology in situ. Despite behaviorally apparent acoustic startle reflexes in humans, the taxonomic equivalent of rodent CRNs has yet to be identified. However, ASR measurements are of major clinical relevance, as altered startle behavior is linked to neuropsychiatric or neurodevelopmental disorders – such as autism spectrum disorders.

Methods: Here, we prepared fixed vibratome-sectioned tissue slice preparations of the cochlear root nucleus as well as whole-mount preparations of the intact peripheral auditory pathway of mice (*Mus musculus*) for immunohistochemical stainings and ethyl cinnamate based tissue clearing. For fiber input tracing experiments, we utilized various genetic reporter mouse models alongside lipophilic tracer dyes. Data were acquired using confocal and lightsheet fluorescence microscopy and analyzed using Imaris software. To probe evolutionary conservation and the functional role of CRNs in various animal models with distinct hearing frequency ranges, we additionally analyzed Mongolian gerbils (*Meriones unguiculatus*), Etruscan shrews (*Suncus etruscus*) and common fruit bats (*Carollia perspicillata*).

Results: In this work, we were able to develop a novel tissue preparation technique that preserves the cochlear root nucleus within its native context in the intact peripheral auditory pathway. Lightsheet images of such chemically-cleared preparations allowed us to

characterize the exact 3D localization, morphology, size and count of the CRN population. Furthermore, we analyzed the afferent and efferent synaptic connectome of CRNs to establish excitatory and inhibitory neurotransmitter identities as well as their respective innervation densities and further plan to probe their role on cellular excitability measures.

Conclusions: In this project, we aimed for a holistic in situ characterization of the CRN population by performing detailed anatomical, morphological and functional analyses regarding cellular location and neuronal connectivity. Our current findings yield strong evidence for distinct CRN subpopulations that may serve differential functions in the ASR pathway – thereby highlighting the surprising complexity of a supposedly simple and rapid reflex circuit.

3. Transient Unilateral Hearing Loss Induces Bilateral but Reversible Central Gain Plasticity

Megan Arnold*¹, Aravindakshan Parthasarathy²

¹*University of Pittsburgh School of Medicine*, ²*University of Pittsburgh*

Background: Unilateral hearing loss (UHL) presents a paradox: despite preserved hearing in one ear, affected individuals often experience disproportionate difficulty with speech perception in noise (SPIN). Standard audiometric assessments do not capture these deficits, suggesting abnormalities in central auditory processing contribute to these impaired outcomes. One proposed mechanism driving SPIN deficits is maladaptive central gain, in which reduced input from one ear drives a compensatory increase in the responsiveness of central auditory pathways. While such plasticity may partially compensate for reduced audibility in quiet, it can impair SPIN by indiscriminately amplifying both target and background signals. Central gain following bilateral hearing loss is well documented in both humans and animal models, but its dynamics in UHL remain poorly understood. Moreover, whether these changes reverse to baseline after hearing restoration has not been systematically examined.

Methods: To address this gap, we developed a reversible model of UHL using unilateral earplugging in Mongolian gerbils, a rodent model with human-like hearing at speech-relevant frequencies. Animals (n=16) were recorded longitudinally at baseline, during earplugging, and after earplug removal. Envelope following responses (EFRs) were measured non-invasively with stimulus parameters emphasizing cortical versus peripheral neural generators. A central gain index was derived as the ratio of cortical to peripheral EFR amplitudes, enabling assessment of level dependence, ear-specific effects, and recovery trajectories.

Results: Earplugging for 14 days produced a stable conductive hearing loss of ~40 dB that resolved upon earplug removal. Peripheral EFR amplitudes were reduced in the plugged ear and unchanged in the unplugged ear. In contrast, cortical EFRs were enhanced relative to peripheral responses, yielding a bilateral increase in central gain: approximately five-fold in the plugged ear and 2.5-fold in the unplugged ear. Central gain enhancement was level dependent, peaking at moderate intensities (40–60 dB SPL). Critically, longitudinal recordings after earplug removal revealed that central gain returned to baseline within two weeks. These findings demonstrate that restoring hearing within this time window enables compensatory gain changes (likely hindering SPIN) to normalize, informing the therapeutic window for intervention in UHL.

Conclusions: These findings demonstrate that UHL drives robust but reversible increases in central auditory gain. The bilateral and time-limited nature of these effects suggests a window

of plasticity during which maladaptive gain can normalize, highlighting the value of early intervention. This gerbil model provides a tractable platform for probing neural mechanisms underlying SPIN deficits and for testing interventions to accelerate recovery of maladaptive plasticity following hearing restoration. Ongoing work examines how central gain dynamics shape neural coding of speech-relevant cues and behavioral outcomes in a tone-in-noise detection task. Together, this work has direct implications for understanding perceptual consequences of enhanced central gain and for optimizing timing and effectiveness of clinical rehabilitation in otolaryngology.

4. Predicting Auditory Midbrain Responses to Natural Sounds With Integrate-And-Fire Gabor Receptive Field Models

Jenna Blain^{*1}, James Dion¹, Ruotong Li¹, Ian Stevenson¹, Monty Escabí¹

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Background: Spectro-temporal receptive fields (STRFs) are widely used to model the time-frequency sensitivity of auditory neurons. Often, STRFs are derived using unbiased synthetic stimuli, such as dynamic ripples or random chords, which can easily be estimated using spike-triggered averaging. However, with natural sounds, decorrelation and regularization techniques are needed to remove embedded stimulus correlations that distort the estimated STRFs. Furthermore, nonlinearities and non-stationarities make it difficult to predict spiking neural responses to natural sounds at millisecond timescales.

Methods: Neural recordings were obtained from the inferior colliculus (IC) of unanesthetized rabbits in response to an ensemble of 50 natural sounds as well as dynamic moving ripples (DMR). We developed a model-based approach for deriving auditory STRFs and predicting single trial spike trains to these sounds. The model consists of a first and second-order nine parameter Gabor STRF (gSTRF; Qiu et al. 2003), accounting for the neuron's linear and non-linear spectro-temporal integration. A four-parameter nonlinear integrate-and-fire (IF) compartment incorporates intrinsic noise, cell membrane integration, and nonlinear thresholding to generate simulated spikes, and a six-parameter adaptive context receptive field that dynamically adjusts the intracellular current to account for slow fluctuations in firing rate in response to naturalistic stimuli. Simulated annealing optimization was used to fit neural data and derive optimal model parameters by minimizing the log-likelihood.

Results: We compared optimal gSTRFs to other approaches such as a generalized linear model and regression. STRFs derived via regression were spectrally smeared, indicating that stimulus correlations were not effectively removed. In comparison, the gSTRF was compact and provided biologically feasible parameter estimates, including best frequency and temporal modulation. The adaptive context receptive field improves predictions of firing changes across sounds not captured by the gSTRF alone. Furthermore, unlike conventional receptive field models, which generate continuous outputs, the model produces and predicts spiking activity with spike timing precision comparable to IC neurons (between ~0.5 -5 ms).

Conclusions: This new approach allows one to derive auditory STRFs and predict neural spiking activity to natural sounds in IC using functionally interpretable basis functions. The small number of functionally interpretable parameters makes exploration of nonlinear and nonstationary effects due to natural sound statistics more feasible.

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5. Layer 6 Corticothalamic Neurons Show Attentional-Gain Responses During a Tone-In-Noise Detection Task

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Background: Neuronal activity adapts to behavioral context, optimizing performance. Although previous studies have shown that layers (L) 2/3 in the auditory cortex show attentional gain when mice are engaged in a detection task, how this modulation is achieved is unknown. Previous studies have reported the presence of noise-invariant neurons in upper cortical layers, and that the amplitude of responses and bandwidth decrease as SNR decreases. Since L6 corticothalamic (CT) neurons play an important role in modulating cortico-thalamo-cortical activity, we hypothesize that this modulation could sharpen the tuning curves and increase the gain of neurons in L2/3 and thalamic nuclei, thereby improving the perception of sound in environments with high background noise, for example. However, it remains unclear whether L6 CT neurons contribute to the mechanisms that improve performance in such conditions. Here, we aim to investigate how L6 CT neurons respond during behavior and whether L6 CT neurons could enhance the perception of target tones embedded in a noisy background.

Methods: We trained adult mice of the Ntsr1-Cre (B6.FVB(Cg)-Tg(Ntsr1-cre)GN220Gsat/Mmucd) line on a fixed hearing background (B6.CAST-Cdh23Ahl+/Kjn) crossed with a reporter line expressing (B6.Cg-Igs7tm162.1(tetO-GCaMP6s,CAG-tTA2)Hze/J) on a tone-in-noise detection task. Constant broadband white noise (50 dB SPL) was presented together with a one-second duration tone of 11.5 kHz (30 to 70 dB SPL), to obtain signal-to-noise ratios (SNRs) of -20 to +20 dB SPL. The mouse had to turn a wheel 25° degrees in either direction to receive a water reward. Mice were then imaged under 2-photon microscopy while performing the task. The pupil diameter was also recorded to assess the animal's attentional state during the task. Passive imaging sessions were conducted after the behavioral sessions to obtain frequency response areas for each neuron.

Results: Our preliminary findings indicate that pure-tone-responsive L6 CT neurons increase their response gain when mice successfully detect a tone embedded in a noisy background, compared to when they do not. This enhancement in response gain appears to become stronger as the SNR decreases.

Conclusions: Our preliminary results are consistent with our hypothesis for a role of L6 CT neurons in hearing in noisy environments. The observed increase in gain of L6 CT neurons at lower SNRs for hit trials in comparison to miss trials suggests that task difficulty and possibly the state of attention may be driving their responses when hearing target tones becomes more challenging.

6. Developing Optimization Strategies to Compensate for Losses Due to Auditory Nerve Synaptopathy

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Background: Difficulty understanding speech in noise is the most common complaint of patients with sensorineural hearing loss. Cochlear neural degeneration (CND)—the loss of peripheral auditory nerve fibers (ANF)—has been hypothesized as a major contributor to these deficits, yet it remains sub clinical in audiometry measurements. In the absence of

noninvasive methods to probe for neural deficits, current hearing-aid (HA) fitting algorithms, such as NAL/NL2, which are based solely on audiometric thresholds, ignore the neural component of hearing loss. The first question we address in this study is how to effectively design speech stimuli that can probe deficits resulting from CND. The second question that we address in this study is how to explore the Hearing Aids parameter space to specifically address neural deficits.

Methods: We used a phenomenological model of auditory periphery (Bruce et. al 2018), to simulate the auditory nerve fiber responses to input sound stimulus. (1) To address the 1st question i.e. the effectiveness of speech materials to detect CND, we developed a Mutual information based framework that uses loss of information between inner hair cells receptor potential and ANFs to quantify the loss of encoding and reduction in information carrying capacity of ANF due to CND. (2) For the second question i.e. Hearing aids fitting, we developed a Simulation-Based Inference (SBI) pipeline to optimize HA level compression algorithms with a focus on compensating for CND. We simulated speech processing under varying degrees of sensory loss and CND. The HA parameter space, including compression threshold, slope, and attack/release times, were systematically explored within the SBI framework to identify parameter sets that minimize neural deficits. Our approach aims to identify alternative parameter combinations that could, in theory, provide better neural compensation and improve speech-in-noise performance.

Results: (1) Our mutual information framework suggests that time-compressed speech (40% and 65% dilatation) produces the greatest sensitivity to CND-related information loss, making it an effective speech probe for evaluating neural deficits. (2) Preliminary results indicate that hearing aid parameters can be explored using SBI to address CND deficits as a framework to reveal parameter-space not captured by the standard NAL/NL2 prescription, particularly for challenging listening conditions.

Conclusions: This project establishes an SBI-based approach to systematically evaluate and optimize HA parameter settings in the context of CND. By highlighting where current gain prescriptions fail, we will re-evaluate compensation strategies to better address the hidden neural deficits underlying speech-in-noise difficulties. Moreover, by exploring the larger parameter space of HA algorithms, this work could uncover fitting strategies that provide more effective compensation for CND-related deficits. [Work supported by MEE SharkTank award and grant R01 DC07910 from the NIDCD of NIH.]

7. Central Auditory Synapse Alterations in Mice With Modified Medial Olivocochlear Efferent Activity

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Background: In many mammals, the auditory system is functionally immature at birth and undergoes postnatal refinement to achieve precise connectivity in adulthood. Spontaneous activity in the immature cochlea drives the organization of auditory brainstem circuits, a process modulated by the medial olivocochlear (MOC) efferent system. While the MOC

pathway is known to protect against noise-induced hearing loss in adulthood, its developmental role remains less understood. We investigated how altered MOC activity during the critical period impacts the synaptic and morphological maturation of the calyx of Held (CH) synapse in the medial nucleus of the trapezoid body (MNTB)

Methods: We combined whole-cell patch-clamp recordings from MNTB principal neurons with 3D reconstructions from serial block-face electron microscopy (SBEM) at P12–14. Three mice genotypes were analyzed: wild type (WT), $\alpha 9$ KI (enhanced MOC activity), and $\alpha 9$ KO (absence of MOC activity). Morphological complexity of CH terminals was quantified using a custom Python pipeline based on graph theory, which enabled classification into distinct morphotypes.

Results: Electrophysiological recordings revealed that $\alpha 9$ KO mice showed electrophysiological responses comparable to WT. However $\alpha 9$ KI mice displayed reduced synaptic strength compared to WT, including smaller excitatory postsynaptic current (WT: 5.01 ± 1.58 nA, $n=28$; $\alpha 9$ KI: 3.61 ± 1.23 nA, $n=25$; $\alpha 9$ KO: 4.23 ± 1.34 nA, $n=20$; ANOVA: $p=0.0013$; Tukey WT- $\alpha 9$ KI, $p=0.0008$), stronger short-term depression during repetitive stimulation (100Hz: WT: $33.59 \pm 1.62\%$, $n=12$; $\alpha 9$ KI: $22.52 \pm 2.99\%$, $n=10$; $\alpha 9$ KO: $33.92 \pm 4.16\%$, $n=11$; ANOVA: $p=0.0224$; Tukey WT- $\alpha 9$ KI: $p=0.0403$) and a decreased readily releasable pool size (WT: 374.02 ± 29.11 , $n=10$; $\alpha 9$ KI: 253.01 ± 21.78 , $n=12$; $\alpha 9$ KO: 323.21 ± 21.39 , $n=9$; ANOVA, $p=0.0057$, Tukey WT- $\alpha 9$ KI, $p=0.005$). Morphological analysis indicated that $\alpha 9$ KI animals exhibited a lower proportion of structurally complex CH terminals and evidence of loss in synaptic pruning, both in pre- and post-hearing stages. The $\alpha 9$ KO mice displayed reduced morphological complexity but neither pruning nor electrophysiological performance were significantly altered (poly-innervation rate: WT/ $\alpha 9$ KO~10%; $\alpha 9$ KI~30%).

Conclusions: Synaptic depression pattern in $\alpha 9$ KI matches well with a higher proportion of simple CHs, according to literature linking structure and function at this synapse. Although previous studies in other auditory centers show both synaptic and morphological alterations when MOC efferent activity is lost, these results suggest that there may be some type of compensation at the level of the MNTB. Our findings demonstrate that enhanced MOC efferent activity during early development triggers profound modifications in both synaptic physiology and morphology at the CH–MNTB synapse. These results highlight the critical role of efferent pathways in shaping auditory brainstem maturation and suggest that altered efferent activity may contribute to long-term deficits in auditory processing.

8. Meclizine, an H1 Antihistamine, Disrupts Roll-Axis Postural Control

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Background: Antihistamines are the largest class of pharmaceutical compounds used to treat and prevent vestibular disorders. Despite their efficacy, we do not know how their ability to suppress vestibular perception affects balance circuits.

Methods: Using a simple vertebrate model, the zebrafish, we show that a commonly-prescribed H1 antagonist, meclizine, modulates balance behavior.

Results: Strikingly, we observed that larval zebrafish swim upside-down during treatment with meclizine, implicating suppression of gravity-driven roll stabilizing postural reflexes. In contrast, H1 agonist exposure caused no change in posture, suggesting a ceiling effect on postural stability. Next, to understand changes to central vestibular processing after

antihistamine administration, we imaged neural responses to body tilts in central vestibular circuits before, during, and after exposure to meclizine. While hindbrain neurons increased their response to roll-axis (leaning left and right) body tilts, preliminary data from downstream midbrain neurons showed changes in stimulus-evoked and spontaneous activity.

Conclusions: These results suggest that antihistamines act directly on central roll-stabilizing circuits. Future work using other drugs will reveal shared and/or divergent mechanisms of antihistamines. We anticipate that our model will generate new hypotheses for clinical studies with the long-term goal of shaping vestibular intervention strategies.

9. Glucocorticoid Receptor Beta Expression as a Marker of Steroid Resistance in Autoimmune Inner Ear Disease

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Background: Autoimmune inner ear disease (AIED) is a form of sensorineural hearing loss that is characterized by either fluctuation or worsening hearing loss in either one or both ears. The first line of treatment for AIED is high-dose corticosteroids. While 70% of initially respond, many lose responsiveness over time. Steroids are the mainstay of treatments as previous trials of other immunosuppressants, such as methotrexate and TNF-alpha inhibitors, have not shown significant benefit. Patients who fail to respond to corticosteroids express high levels of IL-1 β (Pathak et al 2011), and an early-stage clinical trial shows that anakinra, a recombinant IL-1 receptor antagonist, improves hearing capabilities in 46-70% of corticosteroid-resistant AIED patients (Vambutas et al 2014, Vambutas et al, 2025). The mechanism(s) underlying this steroid resistance remain poorly understood. Evidence from other steroid-resistant inflammatory diseases such as asthma and ulcerative colitis suggests the involvement of glucocorticoid receptor beta (GR- β). Glucocorticoid receptor alpha (GR- α) is the active glucocorticoid receptor isoform that mediates anti-inflammatory effects, while GR- β is a splice variant that does not bind glucocorticoids and inhibits GR- α 's transcriptional activity, contributing to steroid resistance. The role of GR- β has not been studied in AIED.

Methods: We examined the relative mRNA expression and protein levels of GR- β and GR- α in peripheral blood mononuclear cells (PBMCs) of patients with AIED using qRT-PCR and Western blot, and investigated how these levels change following treatment with dexamethasone and anakinra under inflammatory conditions.

Results: In PBMCs from corticosteroid-resistant AIED patients, the relative expression of GR- β appears to increase with dexamethasone treatment when co-stimulated with LPS, while treating with anakinra and LPS reduces GR- β expression. Furthermore, this change was also reflected when assessing intracellular GR- β protein with Western blotting.

Conclusions: Characterizing the role of GR- β expression can deepen our understanding of steroid resistance in AIED patients and better predict who may respond to steroids. Further studies are underway to characterize the effect of interleukin-1 expression on GR- β .

10. The Influence of Spectrum and Modulation Statistics on the Neural Representation of Speech in Natural Background Sounds

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Background: Separating target sounds from background noise is challenging for people with normal hearing, and especially challenging for people with hearing impairments as well as artificial speech recognition systems. Why different natural backgrounds mask the perception of speech to varying degrees, and how the brain separates speech from background noise is not yet fully understood.

Methods: Here, we recorded multi-unit activity from the inferior colliculus of head-fixed unanesthetized rabbits passively listening to speech mixed with eleven natural backgrounds (speech babble, bird babble, water, fire, city noise, etc.). Speech was mixed with each original (OR) background at multiple acoustic signal-to-noise ratios (aSNRs). We also presented speech mixed with spectrum-matched (SM) backgrounds that retain the original spectra but have whitened modulations, or modulation-matched (MM) variants that retain the original modulations but have whitened spectra. Using a cross-spectrum shuffling procedure, we separated the foreground- from the background-driven neural responses. The neural signal-to-noise ratio (nSNR) was then estimated and used as a metric of speech coding fidelity.

Results: We find that nSNR depends on background category and statistics. Compared to OR backgrounds, nSNR can increase or decrease for SM and MM backgrounds in a category-dependent manner. This suggests that the original spectra (OR vs. MM) or modulations (OR vs. SM) improve or worsen the neural representation of speech. Interestingly, the impact of background category and statistics was largely independent of aSNR, since increasing aSNR amplified nSNR approximately linearly. We also demonstrate how the background spectrum and modulation statistics affect neural encoding of speech at temporal modulations below 10 Hz, which overlap and potentially mask temporal fluctuations for phonemes, words, and syllables. Spectrum and modulation masking effects were segregated by background category, and a model of the cochlea and auditory midbrain showed similar segregation. Finally, human perceptual tasks using the same backgrounds suggest that nSNR correlates with speech recognition accuracy.

Conclusions: Collectively, the results posit that spectrum and modulation statistics critical to speech masking are reflected in the population activity of the inferior colliculus. These neural response statistics reflect the coding fidelity for speech and likely contribute to downstream signaling underlying speech perception in real-world noise.

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11. A Model of Speech Recognition Reproduces Signatures of Human Speech Perception and Reveals Mechanisms of Contextual Integration

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Background: Humans rapidly and dexterously transform acoustic waveforms into linguistic representations despite the inherent variability of the speech signal. The mechanisms that enable such robustness remain poorly understood, in part due to the lack of stimulus-computable models that both approach human performance and can test theoretical principles and mechanistic hypotheses.

Methods: We built an artificial neural network model of continuous speech recognition optimized to produce sequences of sublexical units (phonemes or characters) from simulated cochlear representations. The front end simulated outer, middle, and inner ear filtering, followed by half-wave rectification and low-pass filtering at 4 kHz to approximate the upper

limit of mammalian auditory-nerve phase-locking, yielding cochleagrams that served as input to stacked convolutional and recurrent layers trained to predict sublexical sequences. The model was trained on 15,000 hours of speech data superimposed on naturalistic noisy backgrounds, with varied SNRs and sound levels.

We compared the model to humans using a new large-scale benchmark of nonword transcription and discrimination along with a battery of previously documented signatures of speech perception, including categorical perception, neighbourhood density effects, phonotactic probability effects, formant-based vowel space, and benefits of talker familiarity. We tested the role and importance of contextual integration by ablating contextual integration mechanisms (recurrent processing), and by manipulating the directionality of contextual processing in the model.

Results: Human nonword transcription and discrimination exhibited highly reliable phoneme confusion structure (split-half $r=0.98$). The model closely matched human performance, reproducing human-like patterns of phoneme recognizability ($r=0.96$) and confusions ($r=0.95$). It also recapitulated classic human perceptual signatures: categorical perception of consonants, sensitivity to neighborhood density and corpus statistics, formant-based vowel encoding, and improved recognition with talker consistency.

Removing contextual processing substantially reduced human–model similarity, suggesting that human recognition depends critically on integrating surrounding speech information.

Conclusions: We built a biologically grounded stimulus-computable model of speech perception, and developed a suite of evaluations with which to compare it to humans and probe the basis of recognition. The model aligns closely with human behavior on speech perception tasks, but only if allowed to integrate information from the surrounding speech signal, implicating contextual processing in human speech perception. By providing both a working model and an evaluation suite, this work supports systematic assessment of existing and future theories of speech perception.

12. Is There a Stream Nearby? Auditory Detection of Water in a Natural Environment

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Background: Water is an essential nutrient for living organisms. Surprisingly, knowledge is lacking about the way human beings process acoustic information conveyed by water in natural settings with their ears and their auditory brain. Our research aims to better understand how humans perceive liquid sounds within natural soundscapes, hypothesizing that water holds a particular perceptual role for human observers due to its ecological importance. To achieve this, we sought to address various objectives: (i) understanding which auditory cues and mechanisms humans use for the detection of water sounds in natural environments, (ii) determining if these cues vary as a function of distance, moments of the day and seasons.

Methods: To address this issue, a Mediterranean stream was recorded at varying distances from a stream segment (hereafter called ‘the source’), times of day (morning, noon, evening), and seasons (spring, summer) in a nature reserve (a forest). Human participants tested online under headphones performed a two-interval forced-choice task in which they had to

determine presence of water. On a typical trial, target and standard stimuli corresponded to acoustic samples recorded over a range of 0-100m distances to the source on a given transect, and samples recorded at 150 or 200m from the source on a different transect, respectively. To eliminate memory effects, a single-trial procedure was used where each participant was tested on a single trial only.

Results: The results confirmed that auditory detection of water depends strongly on distance to the source, consistent with expectations from spherical spreading and masking effects produced by the ambient-noise field. Seasonal changes associated with variations in sound-propagation conditions and masking effects produced by geophysical events and animal vocalizations also influenced water detection, whereas diel variations had no significant effect on auditory judgments. The empirical psychometric functions were compared to simulated psychometric functions obtained by running the same task with the same stimuli with a computational model of the auditory system. This model implemented a cochlear filterbank followed by a modulation filterbank and a decision stage based on a template-matching strategy. The best fit to the human data was obtained when the auditory model based its decisions upon skewness and kurtosis of envelope fluctuations at the output of cochlear filters, and cross-channel correlation patterns at the output of modulation filters.

Conclusions: These results suggest that human listener detect presence of water in natural settings by analysing the sparsity and coordination of amplitude-modulation cues conveyed by natural soundscapes, consistent with previous work demonstrating a role of these texture statistics in water identification (McDermott and Simoncelli, 2011).

13. Processing of Salient Vocalizations in Auditory Cortical Regions Cell Types

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Background: Mice produce a rich repertoire of complex vocalizations across diverse behavioral contexts, such as mating and stress. Playback of these calls elicits distinct behavioral responses and neuromodulator release patterns that vary with the listener's state and the context in which the calls occur. The auditory cortex is thought to relay information about these vocalizations to motor and limbic centers, thereby shaping context-appropriate reactions. However, how such salient vocalizations are represented across cortical subpopulations, and how this representation depends on behavioral state, remain unclear.

Methods: We measured vocalization responses in multiple auditory cortical cell types, including pyramidal excitatory neurons and a subset of inhibitory interneurons expressing Neuron-Derived Neurotrophic Factor (NDNF). These interneurons receive strong neuromodulatory inputs and are thought to modulate cortical activity in a state-dependent manner. Using transgenic mice expressing GCaMP in either pyramidal neurons or NDNF interneurons, we performed widefield and two-photon calcium imaging across auditory cortical regions in awake, head-fixed mice. Mice listened to playback of vocalization syllables recorded during negative (stress), positive (mating), or neutral (isolation) contexts, while we simultaneously monitored pupil dynamics and locomotion.

Results: Vocalizations evoked robust, widespread responses across primary auditory cortex (A1) and multiple higher-order regions. Two-photon recordings revealed region- and cell

type-specific response patterns, with subsets of L2/3 and L4 pyramidal neurons, and NDNF interneurons displaying strong selectivity for specific vocalizations or call classes (e.g., mating versus stress). Notably, many neurons responded robustly to natural vocalizations but not to synthetic sounds with matched spectral content, and small ensembles of vocalization-selective neurons could reliably decode call identity. Ongoing analyses are evaluating how behavioral state modulates the selectivity of these responses.

Conclusions: Together, these findings suggest that auditory cortical circuits encode behaviorally relevant vocalizations in a region- and cell-type-specific manner, and highlight a role for NDNF interneurons in shaping state-dependent processing. By identifying circuit mechanisms for encoding natural communication sounds, this work advances our understanding of auditory perception and lays a foundation for future studies of how internal state and experience shape communication.

14. Effects of Cochlear Synaptopathy on Single-Unit Cochlear Nucleus Responses in the Budgerigar

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Background: Cochlear synaptopathy (CS) is a commonly occurring pathology in aging humans, defined as the loss of synapses between hair cells and auditory-nerve fibers (ANFs). CS is hypothesized to impair perception of sounds in noise, but underlying neural mechanisms are unknown. Recent studies suggest that CS can amplify auditory-nerve onset responses and alter temporal dynamics. However, effects of CS on brainstem encoding remain less explored. The current study quantified effects of CS on neural encoding in the nucleus angularis (NA) of the budgerigar, a parakeet species. NA is one of two avian brainstem cochlear nuclei receiving direct input from the auditory nerve. Budgerigars were selected based on recent studies of kainate-induced CS in this species and previous behavioral research demonstrating performance comparable to humans for various complex auditory discrimination tasks.

Methods: The current experiment compared basic NA response properties including frequency tuning curves, post-stimulus time histograms (PSTHs), and rate-level functions between control and CS-induced budgerigars. In CS animals, substantial (60-70%) ANF loss was induced via intracochlear infusions of 1-2 mM kainic acid. Distortion-product otoacoustic emissions were used to confirm normal hair-cell function. For in-vivo recordings of NA units, a craniotomy was made above the anterior, vertical semicircular canal. A glass electrode with 40 to 80 M Ω impedance was placed in the flocculus of the cerebellum and driven into NA to record single-unit responses. PSTHs were obtained with a characteristic-frequency (CF) tone presented 30 dB above threshold. Rate-level functions were created by randomly presenting CF tones of varying sound levels (0 to 80 dB SPL).

Results: NA neurons in control budgerigars had primary-like, onset, and chopping PSTHs in response to CF tones. Tuning curves were V-shaped, with characteristic frequencies within the range of the budgerigar's behavioral sensitivity (i.e., up to 5-6 kHz). Strength of synchrony was calculated from PSTHs using vector strength (VS). VS was greatest below 1 kHz and generally insignificant above 2 kHz, in contrast to budgerigar ANFs which show significant VS up to 4-5 kHz. From rate-level functions, we found that some NA units monotonically increased firing rate as sound levels increase, while others had nonmonotonic rate-level functions. NA units in CS-induced animals showed the same PSTH response categories and had broadly similar tuning curves, VS of phase-locking, and rate-level

functions to control units. Population results are compared between groups to quantify changes in NA encoding with CS.

Conclusions: Preliminary results suggest that NA units in the CS-induced budgerigars have similar characteristics to control units. Further analyses will test population differences and characterize potential changes in onset activity, adaptation, and envelope encoding in CS-induced animals. Research in this species can provide important insight into the neural bases of normal and hidden hearing loss.

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15. Neural Correlates of Decreased Sound Tolerance Disorders: Insights From Task-Based fMRI Study

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Background: Sounds that do not bother most people—a neighbor chewing, a pen clicking, or the hum of machinery—can be intolerable for individuals with decreased sound tolerance (DST) disorders. DST includes clinically distinct but often co-occurring disorders, most notably hyperacusis and misophonia. Hyperacusis involves increased sensitivity to sounds above a certain loudness threshold, causing discomfort or pain. By contrast, misophonia is characterized by intense negative emotional reactions to specific “trigger” sounds (e.g., chewing or sniffing), regardless of their volume. Despite growing clinical recognition, the neural mechanisms underlying these disorders remain poorly understood. Prior neuroimaging studies have typically examined hyperacusis and misophonia in isolation and without accounting for comorbidity. To address these gaps, the present study aims to delineate the neural correlates of hyperacusis, misophonia, and their co-occurrence using task-based fMRI with emotionally valent sounds.

Methods: Ninety-two young adults were recruited and categorized into four groups: misophonia only (MISO; n = 29), hyperacusis only (HY; n = 14), comorbid misophonia and hyperacusis (MiHy; n = 24), and age- and sex-matched healthy controls (CTR; n = 25). Participants completed a task-based fMRI experiment during which they listened to 90 emotionally valent sounds (30 unpleasant, 30 neutral, and 30 pleasant) from the International Affective Digitized Sounds-2 database. Participants rated each sound as pleasant, neutral, or unpleasant using button presses. Whole-brain and region-of-interest functional activation, as well as seed-to-voxel functional connectivity analyses, were conducted with a primary threshold of $p < 0.001$ and cluster-level family-wise error correction at $p < 0.05$.

Results: Behaviorally, the comorbid group (MiHy) rated more sounds as unpleasant than both MISO and CTR groups, suggesting heightened aversive perception. fMRI analyses revealed both distinct and overlapping neural patterns. MISO and MiHy (relative to CTR) showed increased engagement of visual processing regions and altered salience–visual network connectivity during unpleasant (versus neutral) sound processing, suggesting atypical cross-modal sensory involvement. The HY and MiHy (relative to MISO and CTR) groups exhibited reduced connectivity between salience hubs and frontal control regions, indicating impaired top-down regulation. Notably, in MISO, this connectivity was preserved for non-trigger unpleasant sounds, suggesting intact regulation in that context. The comorbid group (MiHy) combined both neural signatures: altered visual engagement and reduced

regulatory control. Finally, both MISO and HY (relative to CTR) groups showed increased dlPFC–SMA connectivity, pointing to a shared mechanism of motor readiness.

Conclusions: Misophonia was linked to atypical cross-modal visual processing, hyperacusis to reduced top-down control, and comorbidity combined features of both, revealing distinct and overlapping neural correlates of DST. Future research should integrate neural and behavioral measures to refine mechanistic models and guide targeted interventions.

16. Aging Masks Auditory Dysfunction Associated With the Autism Spectrum Disorder Gene *Mef2c*

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Background: Autism spectrum disorders (ASDs) are associated with sensory deficits. Specifically, difficulties in hearing and sound processing are often reported in both children and adults with ASD. Mutations or deletions in the *MEF2C* gene results in a syndromic form of ASD known as *MEF2C* haploinsufficiency syndrome (MCHS). Global *Mef2c* heterozygosity is sufficient to elicit MCHS-like phenotypes in mice, including sensory deficits. Young-adult *Mef2c*-heterozygous mice exhibit auditory nerve functional impairment and a moderate reduction in hearing sensitivity. To date, most studies of auditory dysfunction in ASD preclinical models have focused on early development and young adulthood. The long-term trajectory of these deficits with advanced age is largely unknown, highlighting a critical gap in understanding the intersection of aging and auditory function in ASD.

Methods: Global *Mef2c*-heterozygous mice were backcrossed to B6.CAST mice to eliminate the *Cdh23Ahl* mutation present in the C57BL/6J background, which causes early-onset hearing loss, for auditory functional assessments. Auditory brainstem responses were used to assess hearing sensitivity and auditory nerve activity in aging *Mef2c*-heterozygous and wildtype mice. Bulk RNA-sequencing of cochlear tissue and Simoa (Quanterix) analysis of peripheral blood were performed to assess differential gene expression and circulating neurodegenerative biomarkers, respectively. Cognitive and behavioral phenotypes relevant to MCHS were evaluated using open field, vertical locomotor, social interaction, and acoustic startle tests.

Results: Aging *Mef2c*-heterozygous mice displayed similar auditory thresholds to wildtype controls, suggesting similar age-related hearing decline. Analysis of suprathreshold auditory brainstem responses revealed no significant differences in wave I amplitude or latency between groups, indicating that both genotypes experience similar age-related reductions in peripheral auditory nerve function. However, aging *Mef2c*-heterozygous mice exhibited a reduced acoustic startle response compared to wildtype controls, suggesting enhanced age-dependent declines in central auditory processing. Behavioral testing revealed increased hyperactivity in aging *Mef2c*-heterozygous mice compared to wildtype controls during the open field and locomotor activity assays, similar to changes reported in young-adult mice. Social interaction with social novelty test showed no significant differences between genotypes, indicating similar social behavior with aging. Bulk RNA-seq analysis of cochlear tissue identified a small number of differentially expressed genes between aging *Mef2c*-heterozygous and wildtype mice. Finally, biomarker analysis using Simoa (Quanterix) technology revealed evidence of increased age-related neurodegeneration in *Mef2c*-

heterozygous mice compared to wildtype controls, including increased levels of neurofilament light chain (NFL).

Conclusions: While young-adult Mef2c-heterozygous mice exhibit auditory nerve impairment and mild hearing loss, age-related declines seem to mask auditory sensitivity, peripheral auditory nerve activity, and cochlear gene expression changes between Mef2c-heterozygous mice and wildtype controls. Persistent hyperactivity and reduced acoustic startle responses, however, highlight central auditory processing effects and underscore the need to investigate how ASD risk genes influence cognitive behavioral outcomes across the lifespan.

17. A Comparison of Auditory Brainstem Responses to Diverse Stimulus Conditions in Rhesus Macaques

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Background: Nonhuman primates (NHPs) are a critical translational model for human hearing, with cochlear mechanics, brainstem and cortical organization similar to those of humans. Crucially, macaques have similar noise exposure susceptibility to humans, and support human-like perceptual and behavioral paradigms, enhancing the translation of both physiological and functional outcomes. In addition, NHPs permit controlled acoustic exposures and post-exposure intervals prior to histology. Together, these features make NHPs uniquely suited for auditory-system studies, particularly of noise injury and its brainstem correlates. However, normative non-invasive physiological measures spanning multiple stimulus classes are scarce in this species. To remedy this, we measured physiological responses to a wide range of stimuli.

Methods: Anesthetized young adult rhesus macaques (*Macaca mulatta*, age 4-7 years, n=16, 8 female, 8 males), were tested using non-invasive physiological assays, including auditory brainstem responses (ABRs) and Distortion Product Otoacoustic Emissions (DPOAEs) in a sound-treated

RF-shielded booth. Anesthesia was induced with ketamine and midazolam and maintained with isoflurane. Eight physiological tests are reported: DPOAEs (1-32 kHz), tones (0.5–32 kHz), chirps and clicks presented at various rates (27.7-200 /s), click and chirp pairs with varying inter-stimulus intervals (1-10 ms). For each, we extracted standard wave metrics (Waves I/II/IV amplitudes and latencies) and quantified ear-wise asymmetries.

Results: Thresholds to tones showed the expected U-shaped frequency dependence, with no sex-related differences. Click and chirp thresholds also showed no sex related differences. Inter-ear threshold differences were ≤ 10 dB SPL for most subjects, with no significant sex- or frequency-related bias. Paired-stimuli conditions exhibited canonical temporal-recovery signatures: reduced response amplitudes and prolonged latencies compared to the first stimulus response at short inter-stimulus intervals, with responses more similar to the first stimulus at longer intervals. Rate-manipulation experiments produced rate-dependent reductions in amplitude and latency increases at higher stimulus rates. Across ears, per-wave response amplitude correlations were stronger for closer tone frequencies and for chirp–click comparisons. Latency correlations were weaker and sparser than amplitude correlations.

Conclusions: Together, these results provide a comparative reference for macaque ABRs across complementary stimulus classes—covering thresholds, ear asymmetries, and temporal-recovery functions—and furnish benchmarks to support longitudinal studies of auditory brainstem physiology in a translational primate model. These data form pre-exposure baselines for chronic noise exposure in the same cohort. By spanning complementary stimulus classes and including DPOAE, this baseline enables sensitive detection of exposure-related changes, including subtle synaptic / neural deficits that may precede threshold shifts, through shifts in wave-I/II/IV amplitudes and latencies, altered temporal recovery functions, and rate-dependent modulation.

18. Probing Invariances in Auditory Event Categorization Using Model Metamers

Hee So Kim^{*1}, Malinda McPherson-McNato², Abigail Noyce¹, Jenelle Feather¹

¹*Carnegie Mellon University*, ²*Purdue University*

Background: Real-world acoustic inputs contain rich sensory information that we parse into discrete auditory objects and categories. However, the transformation from acoustic waveforms to neural representations is complex and poorly understood, especially as neural representations are invariant across some feature changes, such as variation in the exact pitches or timing. Further, previous investigations of auditory category representations have largely explored speech, music, or tone structures, with very few studies exploring categorical representations of non-speech stimuli. Computational modeling allows researchers to explicitly probe the transformation from input waveforms into higher-level representations, with recent work suggesting that deep neural networks (DNNs) are well-suited to model human auditory processing. Here, we test whether the invariances of DNNs trained to categorize non-speech sound stimuli are aligned with the invariances of human observers.

Methods: We developed an experimental paradigm to compare humans and DNNs using forced-choice classification across 25 non-speech sound categories using sounds obtained from the FSD50K dataset. For the tested models, we generated ‘model metamers’—stimuli whose activation produces the same response as the natural stimulus at a given model stage. We then tested whether humans (n=4) could recognize the model metamers as well as they could recognize the original audio. Recognizable model metamers imply that invariances of the model are shared with those of human observers. We tested DNNs pretrained on three different tasks: (1) word recognition only, (2) auditory event recognition only, and (3) a multi-task model for word recognition, speaker recognition, and auditory event recognition. These models were trained to be robust to small “adversarial” perturbations in the input cochleagram, a technique that was previously shown to help align the invariances of models with the invariances of human observers in the speech domain. For comparison of including this type of training, we also tested a multi-task model without adversarial training.

Results: First, humans and models can reliably categorize the natural sounds, demonstrating that our novel behavioral paradigm is well suited for testing invariances in auditory categories. For all models, we found that recognition accuracy for model metamers dropped significantly, but there was variability across models. Metamers generated for the adversarially trained auditory event recognition model were the most recognizable among the four models. This result indicates that task-specific optimization plays a significant role in creating human-like invariances. Among the multi-task models, metamers from the

adversarially trained model had significantly higher accuracy, confirming that training with adversarial perturbations helps align model invariances more closely with those of humans.

Conclusions: The results indicate that model metamers provide a powerful tool for probing human auditory perception in the domain of categorical representations of auditory events. These findings provide additional evidence that adversarial training and task-specific optimization can improve model alignment with human perception.

19. Detecting Individual Auditory Brainstem Responses (ABRs)

Malcolm Slaney¹, Emily Kuo^{*1}, Daibhid O Maoileidigh¹

¹*Stanford University*

Background: The Auditory Brainstem Response (ABR) is a measure of neural activity in response to sound stimuli. It is widely used for diagnosing hearing loss in humans and other animals. Current practice estimates hearing thresholds by identifying peaks in the mean ABR over hundreds of trials. However, thresholds based on the mean ABR alone decrease without bound as the number of trials used to estimate the mean increases.

We propose to score the similarity of each ABR trial with the population of ABR trials using covariance. By comparing the score distributions of stimulus-present and stimulus-absent trials, we define an ABR threshold based on the discriminability index d' . We show this measure converges as the number of trials increases.

Methods: We build a model to assess our new method, compare our new method with conventional methods, and confirm the theoretical basis for our method with a simple model and simulations.

We simulate ABR trials by generating deterministic waveforms with added Gaussian white noise. Increasing the amplitude of the deterministic waveform mimics responses to sounds of increasing stimulus level.

We then filter the simulated trials and calculate their mean for each stimulus level. At a given level, each individual trial is assigned a score equal to its covariance with the mean. The score distribution quantifies similarity between the trials.

Lastly, we compute the discriminability index d' value between the score distributions of a stimulus-present condition (non-zero waveform amplitude) with that of a stimulus-absent condition (no deterministic waveform). Because d' is the distance between the distributions, a stimulus is detected when d' is sufficiently large (for example, $d' > 1$). The stimulus level at which stimulus is detected is defined as the ABR threshold.

Results: We show that d' converges to a constant value as the number of trials increases. Consequently, ABR thresholds based on d' no longer decrease without bound as the number of trials increases. Using our simple model, we show how d' is related to the signal-to-noise ratio.

Conclusions: Previous ABR methods use the mean ABR waveform alone, causing the ABR threshold to depend on the number of stimulus trials. In this study, we introduce an alternative approach that uses covariance and d' to detect ABRs. This method defines an ABR threshold that becomes independent of the number of trials as the number increases. Future work will assess the utility of this approach using human and animal ABR data.

20. Effects of the Vestibulocochlear Implant on Balance and Gait in Bilateral Vestibulopathy

Meichan Zhu*¹, Rik Marcellis², Paul Willems³, Jona Beckers², Bernd Vermorken¹, Benjamin Volpe¹, Stan van Boxel¹, Joost Stultiens¹, Elke Devocht¹, Angélica Pérez Fornos⁴, Nils Guinand⁴, Kenneth Meijer³, Raymond van de Berg¹, Christopher McCrum³

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Background: Bilateral vestibulopathy (BVP) often causes a range of debilitating symptoms. The resulting gait and balance problems significantly increase the risk of falls. The vestibulocochlear implant (VCI) is a neuroprosthesis that may offer a promising treatment for patients with severe BVP. This study aimed to explore the impact of different vestibular stimulation modes on gait and balance outcomes.

Methods: The VertiGo! trial is a prospective, nonrandomized, single-center cohort study. The trial includes nine patients with severe BVP who demonstrated inadequate compensation despite conventional treatment. Nine patients received the VCI and were extensively tested in four testing weeks, the first providing reference data (no VCI stimulation) followed by three weeks each with different stimulation modes: (A) baseline stimulation combined with head motion-modulated stimulation, (B) reduced baseline stimulation combined with head motion-modulated stimulation, and (C) only baseline stimulation, without modulation. For the gait and balance assessments, patients walked at 0.6m/s, 0.8m/s, and 1.0m/s on an instrumented treadmill integrated in a 6-DoF motion platform (CAREN; Motek). Five walking conditions were evaluated: unperturbed walking in both lit and dark environments, along with three sizes of pseudorandom mediolateral platform sway perturbations. Coefficients of variation (CoV) were analyzed using 3D motion capture data for step time, step length, double support time and step width. Patients also completed the Mini-BESTest each week.

Results: Step time, length and double support time CoV consistently reduced with increasing walking speeds across all walking conditions whereas step width CoV increased with increasing walking speeds across all walking conditions. Larger perturbations increased CoV consistently. The CoV in the reference mode and stimulation modes A, B and C varied across patients for all gait parameters with no consistent effect for any one stimulation mode (e.g., 5 of 9 patients had lower step time CoV and step length CoV for one stimulation mode but these did not always align with the modes with lower CoVs in the other parameters and the stimulation mode that benefited one patient was often not the mode that benefited another patient). Darkness usually increased CoV but no clear effect of a specific stimulation mode in darkness was found. In the Mini-BESTest, at least one stimulation condition total score was higher than the reference total score for 6 of 9 patients but these were distributed across the different stimulation modes.

Conclusions: Walking speed, perturbations and stimulation modes can influence gait variability and balance performance. While specific stimulation modes may provide slight advantages during acute stimulation, individual responses exhibit considerable variability. The findings emphasize the importance of further investigating tailored stimulation. Investigation into potential differences between acute stimulation and ongoing stimulation is needed.

Poster Blitz Session II

4:45 p.m. - 5:45 p.m.

Meeting Room 209ABC, Floor 2

21. Single-Cell Proteomics and AP-MS to Elucidate Auditory Mechanotransduction in Mammalian Cochlea

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Background: The mammalian cochlea contains two mechanosensitive receptor cell types, inner hair cells (IHCs) and outer hair cells (OHCs), essential for hearing. Despite their importance, the molecular mechanisms driving their distinct function, particularly those underpinning mechano-electrical transduction (MET), remain incompletely understood. This study is the first effort to generate distinct proteomic profiles for IHCs and OHCs, using postnatal day 7–9 (P7–P9) murine cochleae, to uncover key proteins involved in auditory signalling, including novel components of the MET complex.

Methods: Due to the limited number and fragility of cochlear hair cells (~3,500 IHCs and ~12,000 OHCs per cochlea), we employed a suction pipette technique to isolate individual OHCs from enzymatically treated, mechanically dissociated tissue. Isolated cells were cryopreserved and processed using a streamlined one-pot protocol that combines lysis, sonication, denaturation, and tryptic digestion in a minimal volume. We applied TimsTOF SCP with Evosep One for mass spectrometry, identifying up to 500 protein groups per single OHC and 2,200 from pooled five-cell samples. Further analysis will use the higher-sensitivity TimsTOF Ultra 2 system.

Results: Initial work focused on OHCs due to their accessibility. Hair cell-specific proteins were successfully identified in OHCs. We now aim to compare them with recently isolated IHCs. To specifically study the MET complex, we integrate single-cell proteomic data with affinity-purification mass spectrometry (AP-MS) targeting the known MET component protocadherin 15 (Pcdh15). As Pcdh15 is an intrinsically disordered protein (IDP), we apply crosslinking strategies and optimized buffers to capture weak or transient interactions. Potentially interesting protein-protein interactions will be validated using AlphaFold3 and AlphaPulldown.

Conclusions: This study provides new molecular insights into cochlear mechanosensation by profiling key proteins and identifying components of the MET complex. Our approach advances single-cell proteomics in fragile cell types and contributes foundational knowledge for therapeutic strategies in auditory disorders.

22. Towards Precision Medicine in Hearing Loss: Genetic Diagnosis and Functional Studies in Zebrafish

Paula Buonfiglio^{*1}, Carlos David Bruque², Mariela Pace³, Lucia Salatino⁴, Vanesa Lotersztejn⁵, Sebastián Menazzi⁶, Paola Plazas⁷, Ana Elgoyhen⁸, Viviana Dalamón³

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Background: Hearing loss (HL) is a highly prevalent condition, affecting nearly 10% of the global population, and genetic factors account for more than half of the cases. Congenital HL occurs in approximately 1 in every 500–1000 births, predominantly as non-syndromic forms (70%), usually with autosomal recessive inheritance (80%). While GJB2 and GJB6 variants represent the most frequent causes, more than 100 genes have been implicated in HL. Whole-exome sequencing (WES) has become a valuable diagnostic approach, allowing simultaneous interrogation of all candidate genes. Nonetheless, the interpretation of novel missense variants remains a major challenge for establishing robust genotype–phenotype correlations. In this study, we investigated the genetic etiology of HL in an Argentine cohort and functionally evaluated novel variants.

Methods: An initial genetic screening of 1,200 HL patients was performed using Sanger sequencing and GAP-PCR for GJB2 and GJB6 genes. In a subset of 100 unresolved cases with moderate HL, STRC deletions were tested using multiplex ligation-dependent probe amplification. Subsequently, WES was applied to 100 families (82 non-syndromic and 18 with additional clinical features, presumably syndromic). In one non-syndromic family, we identified a novel MYO6 missense variant, which was further characterized by AlphaFold2 protein modelling, followed by in vivo functional validation through zebrafish knockdown phenotype rescue assay.

Results: The GJB2/GJB6 analysis yielded a diagnostic rate of 15.5% in sporadic cases and 36% in familial cases. Screening for STRC deletions accounted for 6% of moderate HL cases. WES provided a molecular diagnosis in 45% of families, with nearly half of the pathogenic variants being novel. Among these, a MYO6 variant segregating with postlingual HL was identified. Structural modeling predicted that this variant markedly altered the protein’s surface electrostatic potential, suggesting a disruptive effect on its function. Functional validation in zebrafish experiments provided further support, as mutant mRNA failed to rescue the knockdown phenotype, confirming its pathogenicity.

Conclusions: This work highlights the power of combining comprehensive genetic testing with in-silico modeling and zebrafish functional assays to strengthen variant interpretation. The diagnostic algorithm applied in this cohort proved highly effective and supports the integration of preclinical models into routine pipelines for hereditary HL, ultimately advancing precision medicine and therapeutic perspectives in the field.

23. Determining Tropism of AAV Serotypes in the Neonatal Mouse Cochlea

Tracy Cheng^{*1}, Alan G. Cheng¹

¹Stanford University

Background: Over 170 genetic mutations have been identified to cause hearing loss, with many amenable to replacement via gene therapy in preclinical models. More recently, clinical trials in gene therapy have revealed promising results for otoferlin-related hearing loss.

Adeno-associated viruses (AAVs) have become increasingly popular as a vehicle to deliver gene replacement for genetic hearing loss because of their high tropism and low immunoreactivity. Currently, there are multiple AAVs reported with different tropisms when injected through the posterior semicircular canal (PSCC). We set out to characterize the tropism of 5 AAVs in neonatal mice after PSCC injection, with the goal of determining one that is most efficient in gene therapy for *Tmprss3* KO mice.

Methods: C57Bl/6 postnatal 3-day-old (P3) mice were injected with 1 μ l of each virus at 5 nl/s through PSCC injections. 5 different viruses were tested: AAV2/8-CMV.EGFP (5.32×10^{13} GC/mL), AAV2/9-CMV.EGFP (7.55×10^{12} GC/mL), AAV2/Anc80L65.CMV.EGFP (1.89×10^{13} GC/mL), AAV2.7m8-CAG-GFP (5×10^{12} GC/mL), AAV-ieK558R-CAG-mCherry-3xFLAG -WPRES (1.33E+13 GC/mL). Each virus was mixed with 1:25 trypan blue (1:25 v:v) to facilitate visualization during injection. Mice were sacrificed 7 days after injection, and immunohistochemistry of whole mounts was performed to determine expression of mCherry or GFP using an anti-GFP antibody. Tissues were also immunostained for inner and outer hair cells (IHCs and OHCs, Myo7a) and support cells (SCs, Sox2). Cell counts of hair cells and support cells were performed in each cochlear turn in whole mounts to compare the transduction efficacy of the different AAVs. Saline injection and the contralateral ear were used as controls.

Results: For AAV2/8-CMV.EGFP and AAV2/9-CMV.EGFP, IHC transduction was most efficient in the apical turn ($60.4 \pm 7.5\%$ and $61.4 \pm 10.4\%$, respectively) with a decreasing gradient towards the basal turn ($35.9 \pm 2.6\%$; $17.6 \pm 3.0\%$). AAV2/8-CMV.EGFP had overall higher transduction of the IHCs, OHCs, and SCs ($46.0\% \pm 4.3\%$, $7.2\% \pm 2.7\%$, $11.7\% \pm 2.4\%$) compared to AAV2/9-CMV.EGFP ($31.2\% \pm 4.3\%$, $6.8\% \pm 3.7\%$ and $5.6\% \pm 4.0\%$). While AAV2/8-CMV.EGFP had minimal transduction efficacy in the contralateral, uninjected ear only in the basal turn ($2.5\% \pm 0.7\%$), AAV2/9-CMV.EGFP had no transduction in the contralateral, uninjected ear. There was minimal toxicity of the AAVs with little to no degeneration of the native IHCs, OHCs, or SCs after injection. Ongoing experiments aim to characterize the transduction efficacy of AAV2/Anc80L65.CMV.EGFP, AAV2.7m8-CAG-GFP, and AAV-ieK558R-CAG-mCherry-3xFLAG -WPRES.

Conclusions: There are significant differences in the transduction efficacy of different AAVs in the IHCs, OHCs, and SCs. The results of this study can guide selection of AAV to deliver gene replacement for *Tmprss3* mutant mice.

24. Responses to Prosthetic Stimulation Remain Robust After Two Weeks of Continuous Ionic Direct Current Stimulation

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Background: The vestibular system in the inner ear detects head motion to maintain balance through reflexes like the vestibulo-ocular and vestibulo-colic reflexes (VOR and VCR). Previous work has shown the advantages of ionic direct current (iDC) over pulsatile stimulation due to its ability to excite, inhibit, and modulate vestibular pathways' sensitivity. Those acute experiments lasted up to 4 hours and used a separated interface nerve electrode (SINE) configuration. Without periodic replacement, that setup would be unsafe for chronic stimulation due to the production of chemical by-products that could alter pH levels at the

tissue beyond safety limits. The effects of continuous iDC delivery to neural tissue have not yet been evaluated for safety. We developed a SINE device to continuously deliver iDC through replaceable gel-filled reservoirs via silicone tubing to fine microcapillary tips in the inner ear.

Methods: We injected both ears in adult chinchillas with the ototoxic drug gentamicin, then implanted microcapillary tips in the anterior semicircular canal of the inner ear's vestibular labyrinth. We used video-oculography (VOG) to measure electrically-evoked VOR (eeVOR) eye movements before and after animals received two weeks of 10-20 μ A continuous iDC using our head-mounted stimulator. The stimulator's electrolytic gel chambers were replaced daily for 2 weeks. During the two weeks of stimulation, VCR head tilt responses to 1-2 second step changes in current amplitude were measured hourly in free-roaming animals.

Results: Sensitivity to iDC stimulation varied across animals, as shown by both eeVOR and eeVCR measurements. Throughout the two weeks of constant iDC stimulation, animals consistently responded with head tilts to hourly changes in current amplitude. Head tilts were roughly aligned with the axis of the implanted semicircular canal and in phase with current changes. eeVOR responses after 2 weeks of stimulation varied but remained robust, with peak velocities exceeding 50 deg/s.

Conclusions: This study demonstrates that delivering ionic direct current to the inner ear over a prolonged period is possible without disrupting physiological function.

25. Identification of Genes That Regulate Development and Maturation of Cochlear Supporting Cells

Sofia Gallino*¹, Matthew Kelley¹

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Background: The sensory epithelium of the cochlea, the organ of Corti, is a highly derived and rigorously patterned structure that acts to convert auditory stimuli into electrical impulses. This structure consists of a highly diverse cellular mosaic that includes two different types of mechanosensory hair cells, and at least six different types of associated supporting cells. While there has been considerable research conducted on the development of hair cells, the maturation of supporting cells, which are equally necessary for normal hearing, has not been sufficiently examined. Therefore, this project aims to improve our understanding of supporting cell (SC) development by characterizing the maturation of pillar cells, Deiters' cells and Hensen's cells during the early post-natal period.

Methods: Cochlear duct cells were dissociated at postnatal day (P) 1, P3, P5, and P7 to obtain individual cells for analysis using single-cell RNA sequencing (scRNAseq) techniques. Bioinformatic approaches, including PySCENIC analysis, were employed to identify potential transcription factors that may modulate the development of different supporting cell types. To validate some of the cell-type-specific genes identified in the data set, we employed in situ immunofluorescent labelling of mRNAs to visualize the localization of candidate transcripts within supporting cell types in cross-sections from postnatal cochlea.

Results: PySCENIC analysis of supporting cell scRNAseq data suggests that *Etv1* and *Etv4* regulons are highly active in inner pillar cells (IPCs) and outer pillar cells (OPCs) during the first postnatal week. This hypothesis is supported by localization of *Etv1/4/5* in inner and outer pillar cells at P1, P5 and P21. Furthermore, the PySCENIC analysis identified

additional transcription factor candidates, such as Gata2, that may play crucial roles in SC development during the same time period.

Conclusions: This study presents a scRNAseq atlas for the development of supporting cells during early postnatal time points. The validation of these results indicates that these data will serve as a valuable resource for the examination of multiple developmental events that occur during the formation of the mammalian cochlea. As a first step in utilizing this resource, I plan to generate a conditional mouse line to disrupt the expression of Etv genes exclusively in postnatal SCs (Sox2CreERT/+; Etv1fl/fl; Etv4^{-/-}; Etv5fl/fl). This approach was designed to ascertain the significance of these transcription factors in the postnatal survival and maintenance of IPC and OPCs. Similarly, a Sox2CreERT/+; Gata2fl/fl model will be established to examine the role of Gata2 in the differentiation and maturation of lateral supporting cell subtypes.

26. Neurotrophin Therapy for Cochlear Synapse Regeneration After Excitotoxic Trauma

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Background: The cochlear synapses between sensory inner hair cells and auditory neurons in the inner ear are vulnerable to noise-induced excitotoxic damage (cochlear synaptopathy). For patients, this is thought to manifest as difficulty interpreting speech in background noise, despite normal hearing thresholds. Although there is growing concern amongst health experts regarding damaging occupational and recreational noise exposure for current and future generations, there are no pharmaceutical treatment options available to patients who experience milder forms of hearing loss. Brain Derived Neurotrophic Factor (BDNF) and Neurotrophic Factor 3 (NT3) are two neurotrophins that target Trk receptors on the auditory nerves, in turn promoting their development, survival, and repair. These two neurotrophins have shown therapeutic potential in cochlear synapse repair in preclinical models of hearing loss. A key challenge to overcome before clinical implementation of neurotrophin therapy is their complicated and costly manufacturing process. We propose the use of novel, Fc fusion variants of these neurotrophins (NT3.Fc and BDNF.Fc), which are produced in mammalian cell lines. This project aimed to assess the bioactivity of our novel BDNF.Fc and NT3.Fc neurotrophins to activate their target Trk receptors and repair cochlear synapses after excitotoxic damage using in vitro assays.

Methods: Trk-NFAT-Bla CHO-K1 cell lines that express high-affinity human Trk receptors for either NT3 or BDNF were used to characterise the dose-response relationship and EC50s (dose that produces half-maximal response) of BDNF.Fc and NT3.Fc for Trk receptor activation. These EC50s were benchmarked to those of commercially available native BDNF and NT3 variants, respectively. Postnatal day 2-5 C57BL/6 mouse cochleae were explanted and cultured in kainic acid to simulate excitotoxic damage seen at the glutamatergic cochlear synapse upon excessive noise exposure. The cochleae were treated with NT3.Fc or BDNF.Fc across a range of concentrations. Cochlear synapses were immunostained and imaged, then synapse density quantified for control and treated explants.

Results: Trk receptor assay results demonstrate that BDNF.Fc and NT3.Fc activate their specific Trk receptors and have a lower EC50 (higher potency) than BDNF and NT3,

respectively (analyses ongoing). Preliminary cochlear explant results suggest that explants treated with BDNF.Fc or NT3.Fc had higher synapse density relative to damaged, untreated control explants (analyses ongoing).

Conclusions: These results show promise for the therapeutic potential of the novel BDNF.Fc and NT3.Fc neurotrophin variants to activate their target receptors and repair cochlear synapses. If the current trend in the data continues, this project could support further preclinical testing using in vivo animal deafness models and first in human clinical trials. Further research is required to understand whether cochlear synapse repair corresponds to improvements in hearing function following excessive noise exposure.

27. Experimental Programming for Cochlear Implants: Dynamically Focused Stimulation Combined With Monopolar Edge Electrodes

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Background: Channel interaction, or activation of overlapping neural populations, is one likely contributing factor to cochlear implant (CI) users' difficulties in understanding speech in noise and music. Clinical strategies in CIs use monopolar (MP) stimulation, which results in broad current spread that contributes to channel interaction. Current focusing with adjacent electrodes is one potential solution to reduce spread. Experimental programs using current focusing to reduce channel interaction have shown potential benefit; however, these programs have only been tested using 14 of 16 electrodes, as the electrodes at the edge of the array have no flanking electrodes to use for focusing and are therefore deactivated. Using these edge electrodes with monopolar stimulation may provide better spectral resolution by reducing channel filter size and fuller sound quality by stimulating spiral ganglion neurons at the edges of the electrode array that were previously un-stimulated in focused programs. In this study, participants were tested on speech in noise and timbre instrument recognition using three programs: a 14-channel monopolar program (MP14), a 14-channel dynamic tripolar program (DT14), and a 16-channel dynamic tripolar program with electrodes 1 and 16 active as monopolar electrodes (DT16).

Methods: At this point, data have been collected from 11 ears in 9 adult participants (mean age = 50.9 years, SD = 19.4 years). All participants used Advanced Bionics devices and were tested across two sessions. In session one, three experimental programs were created: auditory detection thresholds and max comfort levels were first measured across all electrodes to be used as the programming levels, which were further fine-tuned using live speech. Participants finished session one by completing the test battery using their clinical device as training. The test battery consisted of the instrument timbre recognition task from the UW CAMP test, closed-set /hVd/ vowel identification in noise, and the closed-set American English Matrix Test (AMT) in adaptive noise. In session two, participants completed the test battery for each program, with program order block-randomized across participants. Data was analyzed using linear-mixed effects models to determine the effect of program.

Results: Planned comparisons showed no significant mean difference between MP14 and DT14 (testing the effect of focusing) and DT14 and DT16 (testing the effect of MP edge electrodes), with high variability across subjects. Despite incorporating additional training during session one and increased practice trials for each program during session two, the timbre task shows significant learning with test order ($\beta = 5.81\%$ /test block, $p = .028$). AMT and vowels were stable across test order.

Conclusions: Reincorporating electrodes 1 and 16 as monopolar electrodes in a dynamically focusing program show no clear benefit in this interim analysis, but data collection is still underway. It is possible that the added interaction from these electrodes contributed to this lack of benefit.

28. Transcriptomic Analysis of Newly Regenerated Avian Auditory Hair Cells

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Background: Inner ear sensory hair cells (HCs) are essential for transducing auditory and vestibular stimuli. In mammals, lost HCs do not regenerate, resulting in permanent sensory deficits. In contrast, non-mammalian vertebrates, such as birds, can regenerate HCs through activation of adjacent supporting cells (SCs). Newly regenerated HCs in the avian hearing organ, the basilar papilla, differ from fully mature HCs by lacking hair bundles and innervation. We hypothesize that the transcriptomic profiles of these nascent HCs are distinct and reflect an intermediate phase of HC identity that precedes full cytomorphological and functional maturation.

Methods: Chicken basilar papilla hair cells were ablated with an injection of sisomicin into the inner ear at post-hatch day 7. We utilized single-cell RNA sequencing to profile the undamaged chicken basilar papilla as well as at 5- and 10-days post-sisomicin treatment (PST). A list of the top marker genes of nascent HCs at different time points was curated. The duration of marker gene expression was determined through qPCR and in situ mRNA detection at various days after the first appearance of nascent HCs at 4 days PST. Ingenuity Pathway Analysis was used to identify signaling pathways activated during nascent HC maturation. To investigate the functional requirement of these pathways, pharmacological inhibitors were infused into sisomicin-treated inner ears during the period of nascent HC maturation.

Results: Single-cell RNA sequencing revealed notable differences in gene expression profiles among undamaged HCs, nascent HCs at 5d PST, and 10d PST. Validation of these distinct marker genes showed that nascent HCs, although formed over several days, exhibit a coordinated onset of a nascent gene expression pattern, followed by a transition toward a gene expression profile indicative of HC maturation. Initial results from signaling pathway inhibition experiments will be presented.

Conclusions: Our findings reveal a unique transcriptomic signature in nascent HCs in the chicken basilar papilla, characterized by the presence of genes not usually expressed by mature HCs. These genes may be required for replacement HC fate commitment and differentiation, and their expression may be specific to regeneration versus normal development during embryonic stages. Understanding the molecular mechanisms of HC regeneration in birds could provide valuable insights for developing reprogramming strategies aimed at inducing HC regeneration in mammals.

29. Characteristics of Dexamethasone Palmitate Lipid Nanoparticle in Protecting Against Kanamycin-Induced Ototoxicity: In Vitro and in Vivo Models

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Background: Drug delivery to the inner ear (IE) remains challenge. Dexamethasone (DEX) is widely used to protect against ototoxicity, but its conventional formulation, dexamethasone sodium phosphate (DSP), shows limited penetration after intratympanic (IT) injection. Nanoparticle-based formulations, such as dexamethasone palmitate lipid nanoparticle (DPLN), may enhance delivery and efficacy. DPLN, a liposomal formulation incorporating dexamethasone palmitate, has shown potent anti-inflammatory effects in chronic inflammation models and is clinically used for rheumatoid arthritis and osteoarthritis. This study compared the protective effects of DPLN with DSP in in vitro and in vivo ototoxicity models.

Methods: HEI-OC1 cells were cultured under permissive conditions and seeded in 96-well plates. After 3 h of serum starvation, cells were treated with kanamycin (5-20 mM) for 1-24 h to induce toxicity. Steroid pretreatment (1-200 μ M, 6 h) was performed prior to kanamycin exposure. Cell viability and ROS levels were evaluated at multiple time points.

For in vivo experiments, adult C57BL/6 mice received subcutaneous kanamycin combined with intraperitoneal furosemide for two consecutive days to induce ototoxicity. IT-DPLN or DSP were administered every other day for a total of four injections. Auditory brainstem response (ABR) thresholds were measured at baseline and 7, 14, and 21 days after insult. Hearing gain was defined as the interaural threshold difference between the IT-injected and contralateral ears.

Results: In vitro, 24-h kanamycin exposure caused dose-dependent cell death, with 20 mM reducing viability by ~70%. With 20 mM kanamycin, steroid pretreatment improved viability, with the maximal protection at 10 μ M DPLN (89.1%) and 100 μ M DSP (90.7%). Notably, DPLN reduced early ROS spikes within 3 h post-kanamycin, reaching a maximum of 1.8-fold of control at 10 μ M and 0.6-fold at 100 μ M, suggesting rapid and sustained antioxidant action. In contrast, DSP-treated cells showed elevated ROS, up to 2.6-fold at 10 μ M and 3.0-fold at 100 μ M in 3 h.

In the in vivo experiments, 400 mg/kg kanamycin with 170 mg/kg furosemide induced complete ototoxicity, producing a hearing loss of 82.5 dB SPL. IT-DPLN demonstrated greater auditory protection than IT-DSP, with hearing gains of 10 dB at 1 week and 16.7 dB at 3 weeks, compared with 6.7 dB at both time points for IT-DSP. Control ear showed minimal recovery.

Conclusions: DPLN exhibits stable antioxidant effects, mitigating ROS elevation and improving cells survival in vitro. Sustained release of active DEX likely underlies enhanced auditory protection in vivo, resulting in greater and longer-lasting hearing gains compared with DSP. The pronounced suppression of ROS by DPLN indicates its potential as alternative for IT-steroid therapy, effectively overcoming the limited penetration and viability efficacy of conventional DSP in the IE.

30. The Role of a Conserved Extracellular Loop in TMC1 Gating

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Background: The transmembrane channel-like (TMC) family is an ancient group of proteins conserved across eukaryotes. During evolution, the mechanosensory channels TMC1 and TMC2 first appeared in the vertebrate lineage; they are expressed perhaps exclusively in vertebrate hair cells and seem to be highly specialized to mediate hearing and balance. In mammals, TMC1 appears to have undergone further specialization to function in its primary role in adult auditory transduction. Yet the molecular mechanism by which force opens TMC1 channels is not well understood. Here, we investigate the role of a conserved extracellular loop between transmembrane helices 1 and 2 (the 1-2 loop) that may contribute to channel gating.

Methods: We first used evolutionary analysis to find residues in TMC1 that were positively selected for and may underly its functional adaptations. We then used structural modeling to predict how these regions may affect overall structure and transduction complex binding partners. To assess functional consequences, we then selectively mutated individual amino acids in TMC1, constructed AAV vectors carrying the mutant sequence, injected AAVs into the cochleas of *Tmc1/2* null mice at P1, and recorded transduction currents from cochlear hair cells in vitro at P8-15.

Results: Evolutionary analyses revealed residues in the TMC1 1-2 loop that were positively selected in mammals. Structural modeling suggested that this loop arches over the extracellular side of the pore, approaching both the auxiliary subunit TMIE and the pore-lining helices TM4 and TM6. Mutating two conserved aromatic residues shifted channel activation towards the closed state without affecting slope, suggesting that these residues participate in biasing the channel to be open part of the time at rest.

Conclusions: A combination of evolutionary probing, structure analysis, and electrophysiology suggest the 1-2 loop has a specialized role in mammalian hearing. Targeted mutagenesis influences channel activation and in following, this loop contains several residues linked to hereditary deafness. Integration of evolutionary, structural and physiological studies provides a holistic platform for investigating residues of functional importance in sensory transduction channels.

31. Mechanisms for Generation and Control of EMREOs (Eye Movement-Related Eardrum Oscillations)

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Background: Accurate information about eye movements is necessary for audiovisual integration because such movements shift the positions of the eyes with respect to the head/ears. We have recently reported an eardrum oscillation, time-locked with eye movement onset and occurring in the absence of outside auditory stimuli, that suggests eye movement information is sent by the brain to the ears (Eye movement-related eardrum oscillation, EMREO; Gruters et al. PNAS 2018; Lovich et al. PNAS 2023). But what is the proximal and neural mechanism producing such eardrum oscillations? The chief suspects are the middle ear muscles (MEMs) and the outer hair cells (OHCs).

Methods: We recorded eye movements and associated eardrum oscillations in rhesus monkeys before and after surgical intervention involving the MEMs -- specifically, stapedotomy and tensor tympani tenotomy. Such transections of the tendons connecting the MEMs to the ossicles are typically done in human patients suffering from otosclerosis or

MEM myoclonus, and the procedures were adapted here for the monkey. The procedures served to eliminate the contribution of the particular MEM in question to the EMREO.

Results: Sectioning MEM tendons did not eliminate the EMREO. Following stapedotomy, the EMREO was reduced in amplitude but still occurred. Following tensor tympani tenotomy, the EMREO was actually increased in amplitude. When stapedotomy and tensor tympani tenotomy were combined, the EMREO still occurred, and was again increased in amplitude. Together these findings suggest the MEMs are not the sole cause of the EMREO, but rather regulate its amplitude likely in a push-pull fashion. These results from animals are largely consistent with related preliminary data in human clinical patients who have undergone similar procedures.

A general caveat to the interpretation of the MEM surgical interventions is that the procedure may change the mechanical load on the eardrum such that interpretations of changes in amplitude of the EMREO signal should be confirmed by additional research. However, that the EMREO continued to exist even after both MEMs were disconnected from the ossicles indicates that there must be an additional source of force contributing to the signal. The most likely such source is the OHC, which could potentially generate this signal by oscillating in concert throughout the cochlea. It is also possible that in the event of OHC dysfunction, the signal can be generated by MEMs acting alone, a possibility not tested here.

Conclusions: We interpret these findings in the context of how eye movements may alter sound localization cues. Recent work has shown that forces applied by the MEMs to the eardrum in a cadaver model could alter both interaural timing and level difference cues in an eye movement-dependent way (Cho et al., Hearing Research 2023).

32. Cellular Control of Functional Tonotopic Gradients in Cochlear Bone

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Background: Tonotopy of the basilar membrane arises from an EMILIN-2–dependent stiffness gradient. The surrounding cochlear bone—the stiffest bone in mammals—is essential for hearing, yet how its material properties are set is unclear. Because classic osteoblast/osteoclast remodeling is suppressed in the otic capsule, we hypothesized that osteocytes regulate cochlear bone quality along the cochlea’s established hearing axes. This builds on evidence that TGF- β /Runx2–dependent matrix calibration is required for normal hearing and that TGF- β inhibition can rescue hearing in Runx2 haploinsufficiency.

Methods: Cochlear bones from 4-month-old female C57BL/6J mice were profiled with a co-registered, multi-scale pipeline: quantitative backscattered electron imaging (calcium content), synchrotron μ CT (tissue mineral density and lacunar morphometry), second harmonic generation (collagen organization), polarization-dependent imaging contrast mapping (hydroxyapatite nanocrystal orientation), and nanoindentation (stiffness). Spatial

proteomics by MALDI-QTOF MSI mapped extracellular-matrix peptides; immunofluorescence localized osteocyte/ECM markers. Bulk RNA-seq compared apical vs. basal halves. Notably, PIC-mapping and MSI were applied for the first time to bone to resolve nanoscale crystal orientation and spatial proteomic gradients.

Results: Cochlear bone properties varied coherently across radial and tonotopic axes. Radially, inner cortex was more mineralized than outer cortex at apex, middle, and base. Tonotopically, the basal turn showed higher mean mineral density than the middle. At the nanoscale, adjacent nanocrystal misorientation increased from apex to base, while collagen alignment decreased basally; stiffness tracked mineral content most strongly in lamellar regions. Lacunae were larger and more elongated apically and smaller/rounder basally and inwardly, consistent with heightened apical perilacunar/canalicular remodeling (PLR). Immunofluorescence confirmed inner-layer enrichment of DMP1, osteopontin, and MMP13. Spatial proteomics identified basal enrichment of TGF- β antagonists (Asporin, EMILIN-1). RNA-seq revealed apical TGF- β pathway enrichment with basal up-regulation of antagonists (e.g., *Aspn*, *Dcn*, *Ltbp4*, *Fmod*, *Cilp*). Together, these datasets define a spatially patterned TGF- β program that directs osteocyte PLR and ECM composition, establishing axis-registered gradients in cochlear bone material properties that mirror functional hearing axes.

Conclusions: Cochlear bone behaves as a patterned, functionally graded material that likely co-tunes with sensorineural tissues to support frequency selectivity and gain. An osteocyte- and TGF- β -based program appears to sculpt these gradients, and a shared toolkit—EMILIN-1 in bone mirroring EMILIN-2 in the basilar membrane—may coordinate tuning across hard and soft tissues, supporting integrated cochlear function. In light of prior evidence that TGF- β /Runx2-dependent matrix calibration is necessary for hearing and pharmacologically adjustable, our findings suggest a working causal model linking bone material quality to auditory function and point to osteocyte PLR and local TGF- β tone as testable, potentially modifiable levers in sensorineural/mixed losses (e.g., cleidocranial dysplasia, otosclerosis).

33. cAMP and PKA Signaling is Essential for Mitotic Hair Cell Regeneration in the Basilar Papilla in Vivo

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Background: Hearing loss is a global health issue with a profound impact on quality of life and currently has no restorative treatments. Mammals suffer from permanent hearing loss due to an inability to regenerate sensory hair cells. In contrast, non-mammalian vertebrates, like birds, regenerate hair cells and restore hearing throughout life, a process driven by supporting cell proliferation. Our prior work identified an essential signaling pathway for supporting cell S-phase entry and hair cell regeneration in vivo after hair cell damage induced by an ototoxic drug, sisomicin. To deepen our understanding, we now explore additional pathways that regulate mitotic quiescence in the undamaged basilar papilla and promote supporting cell proliferation after hair cell ablation. Previous in vitro studies found that cyclic AMP (cAMP) activators stimulate proliferation in undamaged auditory sensory epithelium, a response blocked by cAMP-regulated protein kinase A (PKA) inhibitors. Our study uses a robust in vivo model to validate these findings.

Methods: In post-hatch day 7 (P7) chickens, cAMP levels were pharmacologically elevated in the damaged basilar papilla by co-infusing cAMP activators with the ototoxic sisomicin into the lateral semicircular canal. cAMP activators were also infused into the undamaged basilar papilla. Supporting cell proliferation was quantified via EdU incorporation 48 hours post-infusion. To assess the role of PKA, we infused PKA inhibitors with sisomicin and measured their effect on supporting cell proliferation.

Results: Elevating cAMP levels with 8-Bromo-cAMP and 3-isobutyl-1-methylxanthine (IBMX) in the damaged basilar papilla resulted in a significant increase in supporting cell proliferation compared to sisomicin treatment alone. Conversely, PKA inhibitors blocked the typical proliferative response in supporting cells observed after sisomicin treatment. Surprisingly, in the undamaged basilar papilla, co-administration of cAMP activators caused only a minimal increase in supporting cell proliferation, as indicated by sparse EdU incorporation.

Conclusions: Our results reveal that the cAMP/PKA signaling pathway is essential for triggering supporting cell proliferation in vivo after damage. Furthermore, while this pathway can modestly promote S-phase entry in the absence of damage, it is primarily an essential component of the proliferative response to injury. Together with our previous work, these findings demonstrate that multiple intracellular signaling pathways are necessary for S-phase entry of basilar papilla supporting cells in response to hair cell loss. Future research must investigate how these distinct pathways interact to coordinate a precise regenerative response that effectively restores auditory function in the avian inner ear. These insights enhance our understanding of sensory hair cell regeneration in birds and lay the groundwork for future translational studies for hearing loss in mammals that utilize signal transduction pathways as potential therapeutic targets.

34. Oto coherence: Interspecies Analysis of Phase Self-Consistency in Spontaneous Otoacoustic Emission

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Background: Spontaneous otoacoustic emission (SOAE) provides compelling evidence of active force generation inside the inner ear, although significant debate exists about the underlying generation mechanisms. SOAE is commonly characterized by peaks in the spectral magnitudes, which occur at frequencies unique to a given ear. This measure is typically computed as an averaged magnitude spectrum that discards phase information. Here, we explore the hypothesis that this phase information reveals pertinent features of SOAE generation beyond what can be extracted from magnitudes alone.

Methods: Our method characterizes the self-coherence of oscillations over time. This “otocoherence” uses a short-time Fourier transform to provide a set of phase estimates as a function of both frequency and time. For each frequency, we calculate the phase accumulation over time periods of some fixed length. The consistency of these phase accumulations is then quantified, providing an otocoherence spectrum: a function of frequency analogous to the magnitude spectrum, but calculated using only phase information from a single SOAE signal. For a fixed frequency, otocoherence is a function of the length of the accumulation time period, typically falling off roughly exponentially as this time increases. Fitting an exponential to this decay allows us to extract a time constant, a process

we repeat for a handful of SOAE frequency centers per subject. These data allow us to characterize the self-coherence of spontaneous emission as a function of frequency across species. Finally, we introduce a class of dynamic windowing methods to address issues associated with the fundamental time-frequency tradeoff.

Results: We analyzed SOAEs recorded from humans, barn owls, and lizards, and found striking interspecies differences in otochherence. Our comparison of the otochherence decay constants with measures of tuning extracted from auditory nerve fibers (barn owl and Tokay gecko) indicated that the method can predict the upper bound of frequency selectivity encoded in the central nervous system. Since our approach does not involve presenting any external stimuli, our results indicate that measures of the spontaneous emissions on their own allow for a noninvasive and stimulus-independent way to infer the degree of cochlear tuning possible.

Conclusions: We report that SOAE phase information provides data that extend beyond spectrally averaged magnitudes alone. A key finding is that the decay in otochherence at a fixed SOAE frequency appears to allow for estimates in peripheral auditory tuning. These methods can readily be employed to re-analyze previously collected SOAE waveforms from a variety of species, making them of broader comparative utility. Further, we suggest how these methods can be extended to reveal new information about SOAE generation. For example, otochherence-based methods can provide a complementary tool to improve the detection of small-magnitude SOAE peaks near the noise floor.

35. Time-Series Transcriptome Analysis of the Older Adult Mouse Cochlea After Noise-Induced Hearing Loss Reveals an Acute Immune Response and Recovery-Associated Pathways

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Background: Noise-induced hearing loss (NIHL) affects approximately 5% of the global population, significantly impairing health-related quality of life, and is of particular interest in the aging population due to its association with an increased risk of cognitive decline. The pathophysiology of NIHL involves a complex interplay of damage to multiple cellular structures within the cochlea. While the pathophysiology of NIHL has been extensively studied in young mice, limited research exists on the molecular mechanisms underlying NIHL in older adult populations. This study provides the first insights into temporal differences in gene expression following noise exposure in older mice with an average age of 17 months.

Methods: We exposed mice to broadband noise of 115 dB sound pressure level (SPL) for 2h and conducted auditory testing before and after noise exposure to confirm the presence of NIHL. Cochleae were collected at 24h, 72h and 1 week post exposure for RNA sequencing (RNA-seq).

Results: Pathway enrichment analysis using the Ingenuity Pathway Analysis (IPA) tool revealed distinct temporal patterns in the cochlear transcriptome. Acute inflammatory pathways were prominently activated at 24h following noise exposure, while pathways associated with collagen degradation, hormone signaling, potassium channel activity and GABA receptor activation peaked at 72h or 1 week after noise exposure.

Conclusions: These findings shed light on the age-specific molecular responses to acoustic trauma, largely consistent with previous experiments in younger mice, and highlight critical windows for cochlear damage and repair. Understanding these temporal patterns in older adults provides information for the development of targeted treatment strategies to mitigate NIHL and its associated risks.

36. Extending the Therapeutic Window: Ndp Overexpression Improves Hearing in an Adult Norrie Disease Mouse Model

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Background: Norrin, or Norrie disease protein (Ndp), is a secreted protein that functions as a Wnt pathway agonist, playing a critical role in cochlear vascularization, hair cell survival, and maintenance of the sensory epithelium. Mutations in Ndp cause Norrie disease, a rare, X-linked recessive genetic disorder characterized by congenital blindness, progressive hearing loss and cognitive decline. Our Ndp-KO mouse model recapitulates key features of human mutations, since it is not a complete null but reflects reported loss-of-function variants observed in patients. Previous studies have shown that Ndp overexpression can rescue hearing in neonatal Ndp knockout (Ndp-KO) mice, but whether this approach is effective beyond the early postnatal period remains unknown and is essential for defining the therapeutic window for intervention.

Methods: We used a conditional mouse model in which Sox2Cre drives tamoxifen-inducible Ndp overexpression (Ndp-OE) in an Ndp-KO background, enabling temporal control of gene activation in Sox2+ cochlear supporting cells. Sox2Cre; Ndp-KO; Ndp-OE mice were divided into treated (tamoxifen injection at postnatal day 30–35) and untreated groups; a control group consisted of Sox2Cre; Ndp-KO mice lacking the Ndp-OE construct. Cochlear function was assessed by auditory brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAE) performed 1 and 2 months of age to assess baseline hearing, progression, and post-treatment effects. Cell morphology, Ndp expression, hair cell integrity and vascularization were evaluated by immunohistochemistry on whole-mount cochleae and cochlear cryosections. Comparisons were made across wild-type mice, Ndp-KO mice, Sox2Cre; Ndp-KO mice and untreated and treated Sox2Cre; Ndp-KO; Ndp-OE mice.

Results: Through comparative review of published human NDP mutations, we determined that the Ndp-KO model reflects clinically reported loss-of-function variants rather than a complete null, establishing its relevance as a model for Norrie disease. ABR testing showed progressive threshold shifts across all frequencies in untreated controls between 1 and 2 months of age. In contrast, tamoxifen-induced Ndp overexpression significantly reduced threshold shifts at the basal-to-mid cochlear regions, where treated mice maintained stable or improved responses while untreated animals declined. Immunohistochemistry verified successful induction of Ndp in Sox2+ supporting cells and revealed improved cellular organization in the organ of Corti, including preserved hair cells. Vascular analyses compared differences between treated and untreated groups. Comparison with a viral-mediated Ndp delivery model demonstrated differences in expression patterns relative to the genetic overexpression approach.

Conclusions: These findings establish the Ndp-KO model as a clinically relevant representation of human NDP mutations and provide the first evidence that inducible Ndp

overexpression at the young adult stage mitigates progressive threshold shifts. Together, these results support the feasibility of Ndp-targeted strategies for delaying or mitigating progressive hearing loss in Norrie disease.

37. Expanding the Genetic and Phenotypic Landscape of Auditory Nerve Deficiency: Results From Analysis of Whole-Exome Sequencing in a Chinese Cohort of 53 Patients

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Background: Cochlear nerve deficiency (CND) is an important etiology of sensorineural hearing loss. While audiological interventions including hearing aid fitting and cochlear implantation are the first-line treatment for these children, outcomes are variable. Therefore, it is particularly important to define the etiology of CND. However, the pathogenesis of CND in humans remains largely unclear, and no known large-scale genetic study has been conducted on CND yet. Among all genetic testing methods, the trio-WES has the unique advantages in the sensitivity of detecting de novo variants (DNM).

Methods: We recruited 53 trio families with SNHL and CND confirmed by MRI findings from the Eye and ENT Hospital, Fudan University between June 2016 and August 2023. Demographic, clinical, and audiological data were collected from electronic medical record, Blood samples from the affected individual and unaffected biological parents were obtained. Whole-Exome Sequencing and variant calling was conducted. De novo variants were filtered out and the deleterious effect of variants was predicted by SIFT scores, REVEL, and CADD scores. Statistical Analysis was performed to investigate the associations between multiple clinical characteristics of patients.

Results: Among 53 CND nuclear families, 68 de novo variants were identified, among which 26 DNMs were considered possibly pathogenic according to several predicting models, including SMARCE1, ITGB1, TRPM8, MCM4, TUBB3, etc. Functional analysis showed that several genes might be linked to the etiology of CND, including developmental process, synapse, regulation of postsynaptic neurotransmitter receptor activity and regulation of synapse pruning. In addition, the phenotypic associations between damaging DNMs and multiple clinical traits were explored. Damaging DNM had a higher ratio in bilateral, inner ear anomaly cases and slightly lower ratio in systemic anomaly patients.

Conclusions: In this study, we identified a large fraction of genes and their potential pathogenic mutations that may be associated with the development of CND in this largest sequencing study of families with cochlear nerve dysplasia to date. Collectively, our results delineate the genomic landscape of Chinese CNDs and identify several potential pathogenic DNMs of CND.

38. Three-Dimensional Analysis of the Effects of Chronic Implantation and Stimulation on Hearing

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Background: Chronic cochlear implantation and stimulation is known to produce a fibrotic tissue response within the cochlea. This fibrosis would be expected to impact residual hearing; however, previous studies have failed to produce a conclusive correlation between the two. We hypothesise this lack of correlation may be due to limitations in conventional histological techniques – analysis from two-dimensional microtome sections, being unrepresentative of the complex response that occurs following chronic implantation and stimulation. We have leveraged recent developments in tissue clearing – a process whereby normally opaque tissue is rendered transparent - and developed a method to allow for three-dimensional characterisation of the cochlear structures and fibrotic response to chronic implantation and stimulation within an intact cochlea.

Methods: Normal hearing guinea pigs (n=4) were bilaterally noise deafened (10kHz 120dB SPL free-field for 1 hour). After 14 days the left cochlea was implanted with an 8-contact intracochlear electrode array and received stimulation (50 μ C/cm²) for 4 weeks from a clinical stimulator and speech processor. Right cochlea served as deafened, unimplanted controls. Hearing assessments (acoustically evoked compound action potentials, aCAPs, 8 – 32kHz) were performed at implantation and at the conclusion of the chronic stimulation period. Cochleae were collected (with implant left in-situ); immunofluorescently stained with markers for cochlear structures (hair cells and spiral ganglion neurons, SGN) and fibrosis (e.g. fibroblasts and macrophages); and cleared in ethyl cinnamate. The whole cochlea was imaged on a light-sheet fluorescence microscope, and images volumetrically rendered and analysed using Imaris software.

Results: Noise deafening resulted in a significant increase of 58dB (SEM=2.3) at 8-32kHz (n=8 ears). In two animals, chronic implantation and stimulation resulted in a 1.5dB increase in hearing thresholds at 1-32kHz. Histological analysis is ongoing; preliminary analysis indicates chronic implantation and stimulation resulted in fibrotic growth, although this did not appear to affect hair cell or SGN survival.

Conclusions: Three-dimensional visualisation of the cochlea offers unprecedented insight into how the fibrotic tissue response to chronic implantation and stimulation can affect hair cell and spiral ganglion neuron survival. In addition, imaging the cochlea with the implant left in-situ allowed for the identification of the fibrosis and cochlear structures in relation to specific electrodes. Using a range of pathophysiological markers, this study demonstrates a novel method that provides an accurate assessment of the cochlea following chronic implantation and stimulation. This improved validation tool will be used in developing treatments to reverse or prevent cochlea damage.

39. Comparative Analysis of Rna Versus Protein Splicing in Dual Aav-Mediated Gene Therapy in a Mouse Model of DFNB9 Deafness

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Background: Pathological mutations in the OTOF gene cause autosomal recessive deafness 9 (DFNB9). Although dual-AAV gene replacement therapy has been shown to partially

rescue the hearing of patients with DFNB9, the therapeutic effects still need further exploration.

Methods: To investigate the impact of different recombination strategies on the efficacy of OTOF gene therapy, we constructed five dual-AAV1 therapeutic agents using RNA or protein splicing principles.

Results: Based on the recombination strategy using RNA splicing, the AAV1-AK system rescued the hearing of *Otof* ^{-/-} mice to 55–70 dB, which outperformed the AAV1-AP and AAV1-TS systems. Based on protein splicing, the AAV1-intein system rescued the hearing of *Otof* ^{-/-} mice to 35–70 dB, which outperformed that of AAV1-AK, and the effects lasted up to 12 months after injection in both newborn and adult mice. The efficacy of otoferlin re-expression and the number and functional restoration of ribbon synapses in the AAV1-intein system were also better than the AAV1-AK system.

Conclusions: These results show that protein recombination is more efficient than nucleic acid recombination for gene therapy in DFNB9. Therefore, this work not only provides data for optimization of DFNB9 gene therapy, but also provides a reference for the delivery of other large genes.

40. Musical Context Influences the Impact of Vocal Boost Manipulations for Cochlear Implant Users

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Background: Music perception and enjoyment remain a persistent challenge for post-lingually deafened adults with cochlear implants (CIs), largely due to the degraded spectral cues essential for melodic processing. Vocal boosting - enhancing the levels of vocals relative to background – has been proposed as a means of improving sound quality and enjoyment in this population. Yet, whether the benefit of vocal boosting derives from greater access to lyrical content within a coherent musical scene, as opposed to increased audibility over a non-musical masker, remains unclear. This study investigates how the perceptual gains from vocal boosting depend on the acoustic nature of the background.

Methods: Fourteen CI users rated the sound quality of stimuli on a 100-point scale. Vocals from ten popular songs were extracted and paired with three types of backgrounds: original instrumentals, off-key instrumentals (+4 semitones), and multi-talker babble. Each vocal-background pair was normalized to have an equal signal-to-noise ratio (SNR), forming the +0 dB SNR condition. Stimuli were then manipulated to create +9 dB (boosted) and -9 dB (attenuated) SNR conditions. An anchor stimulus (1,000 Hz low-pass filter with white noise) was included. Participants rated stimuli relative to the +0 dB SNR condition for each background. A subsequent forced-choice test assessed recognition of the original song from the three background types.

Results: Background type had a dominant impact on sound quality ratings ($\eta^2=0.68$), exceeding the vocal level effect ($\eta^2=0.35$). Vocals over both original and off-key instrumental backgrounds were rated similarly (58.2 vs. 56.9; $p=0.774$) and significantly higher than vocals over babble noise (22.9-point difference, $p < 0.001$). Thus, CI users reliably differentiated musical from non-musical backgrounds, but were insensitive to large pitch distortions. Vocal boost (+9 dB) increased ratings modestly (+ 7.2 points; $p < 0.05$), while attenuation (-9 dB) produced a more pronounced decline (-14.7 points; $p < 0.001$). Results

revealed a significant interaction between background type and vocal level ($F(2,39)=11.31$, $p < 0.01$), with vocal boost effects varying by background type: babble showed the largest improvement (+16.6 points, $p < 0.001$), followed by original instrumental (+7.2 points, $p=0.001$) and off-key instrumental (+4.1 points, $p=0.122$). Overall song recognition accuracy was 68%. Participants with a history of musical training showed a significant advantage in recognition (75% vs. 61%, Cohen's $d=0.31$) and derived greater benefit from vocal manipulations.

Conclusions: While vocal boosting provides benefit across all background types, the highest absolute sound quality ratings emerge when vocals are embedded within a coherent musical structure, highlighting the importance of preserving contextual cues rather than simply amplifying speech. The ability to distinguish musical from non-musical content, coupled with insensitivity to pitch inaccuracy, underscores a reliance on structural over spectral fidelity. These findings suggest that future CI musical processing strategies should retain musical scaffolding, while selectively boosting vocal prominence to meaningfully improve musical enjoyment.

41. Connexin30-Deficient Mice Increase Susceptibility to Noise via Redox and Lactate Imbalances

Jifang Zhang^{*1}, Jun Yang¹, MaoLi Duan²

¹Shanghai Jiao Tong University School of Medicine, ²Karolinska University Hospital

Background: Noise significantly contributes to one-third of the global burden of hearing loss. The intricate interplay of genetic and environmental factors impacts various molecular and cellular processes that lead to noise-induced hearing loss (NIHL). Defective connexin 26 (Cx26) and connexin 30 (Cx30), encoded by Gjb2/Cx26 and Gjb6/Cx30, respectively, are prevalent causes of hereditary deafness. However, the role of Cx30 in the pathogenesis of NIHL remains unclear.

Methods: Cx30-deficient mouse models related to NIHL were established and subjected to audiological follow-up using auditory brainstem response (ABR) measurements. Cochlear morphology was analyzed via immunofluorescence, transmission electron microscopy, and toluidine blue staining. Changes in oxidative stress were observed and compared using dihydroethidium (DHE), 4-hydroxynonenal (4HNE), and western blot assays. Cochlear glucose transport and energy metabolism were assessed via 2-NBDG glucose uptake and ATP release assays. Metabolomic analysis was employed to investigate mechanisms underlying cochlear energy alterations.

Results: We observed that homozygous Cx30 knockout (Cx30 KO) mice exhibited poorer hearing recovery after noise exposure (97 dB mean sound pressure level for 2 h) and increased susceptibility to noise. In addition to the exacerbation of noise-induced damage to hair cells and synapses, Cx30 KO mice exposed to noise exhibited increased oxidative stress. The 2-NBDG assay showed a reduction in glucose levels associated with a decrease in gap junctions as well as a reduction in adenosine triphosphate release. Glucose metabolomics analysis further revealed that Cx30 KO mice had elevated lactate and NAD⁺ levels after noise exposure, thus worsening anaerobic oxidation from glycolysis.

Conclusions: Our study emphasizes that Cx30-deficient mice increase susceptibility to noise via redox and lactate imbalances in the cochlea.

NIDCD Workshop: Navigating NIDCD Funding for Early Stage and New Investigators

7:00 p.m. - 8:30 p.m.

Meeting Room 209ABC, Floor 2

Kelly King, *NIDCD, National Institutes of Health*

The *Early Stage/New Investigator* workshop will provide practical information on how grant applications are processed within NIH/NIDCD. Specific information will be presented regarding funding opportunities for early-stage investigators, including the NIDCD Career Development Award (R21) and recent changes in NIH policy, providing information to early-stage investigators to facilitate their successful transition from trainee to independent investigator.

NIDCD Workshop: NIDCD Extramural Clinical Trial Workshop

7:00 p.m. - 8:30 p.m.

Ballroom B, Floor 3

Trinh Ly, *NIDCD/NIH*

Castilla McNamara

This workshop will provide investigators an overview of how NIDCD approaches extramural clinical trials. The goal of this session is to help investigators determine clinical trials risks, select the most appropriate funding opportunity as well as highlight important application requirements and points to consider when planning an NIDCD clinical trial. Investigators will also gain an understanding of how NIDCD manages clinical trials and expectations for the implementation of NIDCD funded high-risk clinical trials.

Memorial Symposium: Symposium on Neural Coding: Bertrand Delgutte's Seminal Research on Understanding Robust Hearing in Challenging Conditions

7:00 p.m. - 9:00 p.m.

Meeting Room 208ABC, Floor 2

Chair: Sridhar Kalluri, *Advanced Bionics*

Co-Chair: Philip Joris, *Katholieke Universiteit Leuven*

Co-Chair: Mitchell Day, *Ohio University*

Bertrand Delgutte - Mentor and Colleague Extraordinaire

Ruth Anne Eatock, *University of Chicago*

BARD Gems

Philip Joris, *Katholieke Universiteit Leuven*

Neural Coding of Pitch: Insights From Single Neurons and Beyond

Yaqing Su, *Dandelion Science*

Bertrand Delgutte's Contributions to Understanding Neural Representations of Speech Sounds

Laurel H. Carney, *University of Rochester*

Binaural In Real Life: Bertrand Delgutte's Contributions to Understanding Spatial Hearing

Mitchell Day, *Ohio University*

Bridging Neurophysiology, Perception, and Modeling: Bertrand Delgutte's Contributions on Neural Coding of Binaural Cues in Cochlear Implants

Yoojin Chung, *Mass General Brigham*

Sunday, February 8, 2026

Podium 3: Benchtop to Bedside and Back: Clinical Otolaryngology and Pathology

8:00 a.m. - 10:00 a.m.

Ballroom B, Floor 3

Moderators: Daniel Sun and Nicole Jiam

Biomarkers of Neuroplasticity in the Perilymph in Prelingual Deafness

8:00 a.m. - 8:15 a.m.

Monika Matusiak^{*1}, Anna Konopka², Monika Ołdak¹, Dominika Oziębło¹, Henryk Skarżyński¹

¹*Institute of Physiology and Pathology of Hearing*, ²*PORT Łukasiewicz Research Network*,

Background: Congenital deafness treatment with cochlear implantation (CI) is a standard therapy with, in overall, very good results, however, in some cases implanted children do not reach the age norm in verbal communication. Molecular biomarkers would facilitate the clinical management of deaf born children, helping in predicting the level of linguistic proficiency a child would achieve after CI. Having known, from our previous studies, that the serum level of matrix metalloprotease 9 (MMP-9) has been identified as a biomarker of neuroplasticity in prelingual deafness it is of great interest to investigate the MMP-9 also in the perilymph, as well as look for another biomarkers in the perilymph. Inner ear liquid biopsy would also help in understanding the molecular environment of the diseased organ in congenital deafness cases.

The objective of the study is to investigate the perilymph proteome, including MMP-9, and blood serum proteome, and to see their relation to auditory development after CI.

We have collected 6 pairs of samples perilymph/blood during the surgical procedure of CI from prelingually deaf children in Institute of Physiology and Pathology of Hearing, Warsaw. The perilymph was sampled by using glass capillary via the round window just before the array insertion.

Methods: Using Mass Spectrometry (MS) protein quality analysis was performed in each perilymph/blood sample. MMP-9 was assessed by Enzyme-Linked Immunosorbent Assay (ELISA). Auditory development of implanted children was assessed by using LittleEARs Questionnaire (LEAQ).

Results: We have detected 1471 perilymph proteins, including MMP-9, out of which 441 were detected only in perilymph and 43 were detected only in blood plasma. Preliminary analyses of correlations between perilymph proteomic profiles, blood plasma and auditory development were performed.

Conclusions: There are correlations between proteome content of the perilymph and auditory development outcomes of cochlear implantation in prelingual cases.

Can Impedance Predict Intracochlear Neo-Ossification and Fibrosis in Cochlear Implant Recipients?

8:15 a.m. - 8:30 a.m.

Nicole E. Tomassi*¹, Abbie Hall¹, Caylin McCallick¹, Alexander Geerardyn¹, Julie G. Arenberg¹, Alicia M. Quesnel¹

¹*Mass. Eye and Ear, Harvard Medical School*

Background: Foreign body responses are a common result of cochlear implantation. Although surgical techniques have changed over the years in attempts to reduce insertion trauma, neo-ossification and fibrosis around the electrode array is still commonly observed. Historically, neo-ossification and fibrosis have been thought to affect the resistivity of electrodes, measured clinically as the level of impedance. Since neo-ossification and fibrosis cannot be easily visualized and quantified in living humans, the relationship between these foreign body responses and cochlear implant impedance levels has been limited to animal and tissue-engineered models. However, post-mortem temporal bone specimens enable high resolution 3-dimensional mapping and quantification of intracochlear fibrosis and neo-ossification, allowing the investigation of these relationships in human cochlear implant recipients. The purpose of this study was to relate neo-ossification and fibrosis volume adjacent to individual electrodes in human implanted cochleae to impedance levels measured during life.

Methods: Human temporal bones from cochlear implant users were histologically processed, sectioned, and digitized. 3D reconstructions of the cochleae and electrodes were generated. The anatomical regions of the cochlea including the scala tympani, scala vestibuli, and scala media (when applicable), as well as the electrode array and areas of neo-ossification and fibrosis, were digitally segmented. The individual electrode locations were mapped using the 3D electrode reconstruction and manufacturer reported intra-electrode distances. The last measured impedance before death was extracted from the clinical records of each temporal bone donor. The amount of neo-ossification and fibrosis was compared to impedance levels using Pearson's correlation coefficient.

Results: We expect a total of 16 donors to be represented in this study. Within these clinical records, impedance levels were higher at the base of the array (likely due to insertional trauma) in 14 donors. Preliminary results in seven completely analyzed temporal bone specimens indicate a direct relationship between neo-ossification and impedance at the group level such that more bone deposition was positively correlated with higher impedance levels ($p < 0.001$, $r = 0.484$). The relationship between neo-ossification and impedance at the individual level varies. In the preliminary dataset, no significant relationship was observed between fibrosis and impedance.

Conclusions: Thus far, a positive relationship has been observed between neo-ossification and impedance at the group level. This approach bridges otopathology and clinical outcomes in humans by quantifying these foreign body responses with high precision and relating them to impedance levels of each electrode.

Distribution Patterns of Cochlear Endosteal Invasion in Otosclerosis

8:30 a.m. - 8:45 a.m.

Tomotaka Shimura¹, Nevra Keskin Yilmaz¹, Sebahattin Cureoglu¹, Meredith Adams¹, Rafael da Costa Monsanto*¹

¹*The University of Minnesota*

Background: Most cases of otosclerosis involve bone remodeling localized to the fissula ante fenestram. In a subset of cases, however, the pathological process extends to the cochlear endosteum, resulting in fibrosis, atrophy, or ossification of the cochlear lateral wall. Owing to the limited resolution of current imaging modalities in delineating intracochlear alterations and the precise interactions between osseous and membranous structures, the spatial distribution and morphological patterns of these lesions remain insufficiently defined. The objective of the study was to characterize the frequency, severity, and site-specific distribution of cochlear endosteal invasion in otosclerosis from a pathological perspective.

Methods: Archival histological slides of human temporal bones from donors with otosclerosis from the Paparella Otopathology and Ear Pathogenesis Laboratory collection (University of Minnesota) were examined. Endosteal invasion was defined as direct disruption of the cochlear endosteum and classified into three grades. Grade I was defined as partial contact with the endosteum accompanied by mild surface irregularity, without spiral ligament hyalinization or stria vascularis atrophy. Grade II was characterized by mild pathological changes of the lateral cochlear wall, whereas Grade III encompassed moderate to severe alterations of the lateral wall and/or the presence of intraluminal pathology. Results were statistically analyzed using chi-square tests.

Results: Among 207 ears from 119 donors, cochlear endosteal invasion was identified in 37 ears (17.9%). The distribution of invasion grades was: Grade I, 21.6%; Grade II, 43.2%; and Grade III, 35.1%. Multifocal invasion was significantly associated with higher-grade lesions ($p < 0.0001$). The lower basal turn had the lowest overall frequency of lesions, with an invasion rate of 53.9% compared to other turns. Notably, however, all 7 cases with basal turn involvement demonstrated severe (Grade III) endosteal invasion.

Conclusions: Cochlear endosteal invasion was present in 17.9% of otosclerosis cases and showed site- and grade-specific variability, with the lower basal turn most often affected by severe lesions. This classification system highlights distinct spatial and morphological patterns that may serve as a standardized framework for future clinicopathological studies and could help guide both the timing of medical treatment (e.g., bisphosphonates) and surgical planning.

Hypoxia-Driven Metabolic Reprogramming in Vestibular Schwannoma

8:45 a.m. - 9:00 a.m.

Mikhail Marasigan*¹, Olena Bracho¹, Bryan Souza¹, Diane Jung¹, Olivia Kalmanson¹, Michael Ivan¹, Carolina Benjamin¹, Vivek Kanamuri¹, Simon Angeli¹, Fred Telischi¹, Pei-Ciao Tang¹, Daniela Frasca¹

¹*Miller School of Medicine, University of Miami*

Background: Cancer cells adapt their metabolism to sustain proliferation and survive under nutrient limitation and hypoxia, promoting growth and therapy resistance. Vestibular

schwannomas (VS) are benign intracranial tumors of the vestibulocochlear nerve that often cause hearing loss, dizziness, and neurological deficits. Although VS express hypoxia-related factors, their metabolic pathways remain poorly defined. Elucidating these mechanisms may uncover metabolic vulnerabilities that can be targeted to improve clinical outcomes for VS patients.

Methods: Primary VS cell cultures were established from patient tumors. In parallel, wild-type (WT) and NF2-mutant Schwann cells (SCs) were generated from human induced pluripotent stem cells. Cells were maintained in proliferative Schwann media at 37°C, 5% CO₂, under oxygen tensions of 1%, 5%, or 21% O₂. After seeding (2,500 cells/well, 384-well CellBIND plates) and cell adherence, media was refreshed and metabolic assays (CellTiter-Glo, RealTime-Glo MT, Lactate-Glo, Glucose-Glo, Glutamine-Glo) were performed at 72 hours. Luminescence was measured, and data analyzed by nonparametric analysis of variance.

Results: ATP levels correlated with viability measurements, with the highest levels observed at 21% O₂, indicating mitochondrial respiration as the predominant means of energy production when oxygen is readily available. All cell types utilized glucose and secreted lactate (byproduct of glycolysis), though VS exhibited the lowest levels overall. Notably, VS cells increased glutamine consumption at 1% and 5% O₂ compared to 21% O₂, suggesting hypoxia-dependent reliance on glutamine metabolism.

Conclusions: VS, WT, and NF2-mutant SCs display distinct metabolic profiles shaped by oxygen availability. Our pilot study shows VS demonstrate limited glycolysis but enhanced glutamine utilization under hypoxia, pointing to metabolic dependencies that may be exploited therapeutically. Targeting these adaptive pathways could inform novel strategies for VS control.

Intraoperative Electrically Evoked Stapedial Reflex Thresholds (eSRT) for Cochlear Implant Programming: A Postoperative Validation Study

9:00 a.m. - 9:15 a.m.

Eric Cunningham^{*1}, Isabelle J Chau¹, Rivka Bornstein², Regina Albinus², Pal Sudhirkumar Shah¹, Katrina Stidham²

¹New York Medical College, ²Westchester Medical Center

Background: Cochlear implants (CIs) restore hearing in severe-to-profound sensorineural loss by delivering electrical stimulation to the auditory nerve. Programming requires determining the lower (T-level) and upper (C-level) stimulation limits, which define the electrical dynamic range. In patients unable to provide reliable behavioral feedback, the electrically evoked stapedial reflex threshold (eSRT) can approximate C-levels and improve programming accuracy. The eSRT correlates with behavioral C-levels, but postoperative testing is limited by middle ear pathology, patient intolerance, or low clinical adoption. Intraoperative eSRT may overcome these barriers and offer an initial programming reference, but volatile anesthetics can suppress the reflex. Whether intraoperative eSRT predicts early postoperative values, particularly at middle and apical electrodes, remains unclear.

Methods: In this prospective cohort study, CI recipients underwent routine intraoperative eSRT testing with Cochlear Americas devices. Exclusion criteria included absence or disruption of the stapedial tendon, anatomic barriers, medical instability, or volatile anesthetic exposure within 10 minutes of testing. A single surgeon measured intraoperative eSRTs visually with volatile

anesthetics withheld. Postoperative eSRTs were measured ~1 month later. Response rates were compared via chi-squared tests with Benjamini-Hochberg correction. Threshold changes were analyzed with paired t-tests, and intra- vs. postoperative correlations assessed using Spearman's rank.

Results: Eighty-four cochlear implants in 77 patients met inclusion criteria, with intraoperative eSRT attempted in 72 ears and postoperative testing in 50. Intraoperative response rates were significantly higher than postoperative rates overall (83.1% vs. 70.9%, $p = 0.0005$), with the highest rates at middle and apical electrodes. Median intraoperative thresholds were significantly higher than postoperative thresholds (21.2 vs. 15.6, $p < 0.0001$), but no significant correlations were observed between the two time points.

Conclusions: Intraoperative eSRT was feasible and yielded high response rates, particularly at apical and middle electrodes. However, intraoperative thresholds did not correlate with early postoperative values and cannot currently predict programming levels. These findings support the utility of intraoperative eSRT as a qualitative intraoperative tool, but further refinement is needed to improve its predictive value for postoperative cochlear implant programming.

Linking Data-Driven Audiometric Phenotypes to Speech Perception in Quiet and Noise

9:15 a.m. - 9:30 a.m.

Varsha Athreya*¹, Jwala Rejimon², Malcolm Slaney¹, Matthew Fitzgerald²

¹*Stanford University*, ²*Stanford University School of Medicine*

Background: The pure-tone audiogram (250–8000 Hz) remains the clinical gold standard for assessing hearing, yet it only partially captures suprathreshold deficits. Here, we apply unsupervised, data-driven profiling to derive phenotypes that reflect both audibility loss and suprathreshold performance (word recognition in quiet and speech-in-noise). This approach refines hearing-loss classifications by capturing natural variability in audiogram shape and severity, and by characterizing speech outcomes within each cluster to support precision audiology. In addition, we will identify specific diseases within each cluster (e.g., Ménière's disease, vestibular schwannoma) to probe disorder-linked audiometric and speech-perception signatures. Finally, we will link these phenotypes to real-world device behavior, specifically the adoption of hearing aids and cochlear implants, to inform targeted fitting strategies and treatment selection.

Methods: We conducted a retrospective analysis of audiological records collected at our academic medical center since 2006, excluding cases with conductive or mixed hearing loss (air-bone gap ≥ 10 dB HL). Using K-means clustering (scikit-learn, Python) on 66,956 unique pure-tone audiograms measured at eight frequencies (250–8000 Hz), we derived distinct, data-driven audiometric phenotypes. To relate these phenotypes to functional hearing, we examined speech-perception performance using Word Recognition in Quiet (WRQ) and QuickSIN (speech-in-noise) measures. Within each cluster, we selected individuals with Ménière's disease and vestibular schwannoma to evaluate relative prevalence and to describe diagnosis-linked speech-perception profiles. Finally, we linked cluster membership and diagnosis to real-world device behavior of hearing-aid and cochlear-implant adoption.

Results: K-means clustering uncovered a set of distinct audiometric phenotypes, highlighting diverse configurations and severities across the cohort. Hearing loss increased with age, most prominently at higher frequencies. Despite similar pure-tone averages, audiograms show age-dependent variability in configuration. As hearing loss increases, the likelihood of sub-optimal word recognition in quiet increases, however, most patients display excellent performance. In contrast, even small amounts of hearing loss, particularly in higher frequencies, worsens speech-in-noise performance. In addition, SPIN performance showed greater individual variability than WRQ scores, demonstrating sizable differences in suprathreshold processing under challenging listening conditions. Ménière's disease tends to show rising configurations in younger patients, possibly linked to recent diagnosis, and flat or sloping losses in older patients. In contrast, vestibular schwannomas more often manifest as sloping losses of variable severity.

Conclusions: These findings emphasize that audiometric thresholds alone cannot capture the full complexity of hearing impairment. Clustering revealed distinct phenotypes tied to age and diagnosis, while differences between word recognition in quiet and speech-in-noise performance suggest that the latter is more sensitive to subtle deficits, reflecting challenges in suprathreshold processing. Incorporating speech-in-noise testing alongside audiometric profiling is therefore essential for both accurate diagnosis and tailored rehabilitation. Together, these age- and diagnosis-specific patterns provide insight into the disease course and may inform more targeted rehabilitation strategies.

Swab-Based Genetic Analysis of HPV for Precise Detection and Tumor Profiling in Head and Neck Cancer

9:30 a.m. - 9:45 a.m.

Ella Jackert*¹, Swar Vimawala², Liyang Tang², Daniel Kwon², Niels Kokot², Uttam Sinha², Albert Han²

¹*Keck School of Medicine of USC*, ²*USC Caruso*

Background: Current methods to detect and genotype human papillomavirus (HPV) in oropharyngeal squamous cell carcinoma (OPSCC) rely on tissue specimens or assays with limited specificity. We validated a minimally invasive swab-based strategy with potential to improve diagnosis, tumor characterization, and surveillance.

Methods: Twenty-five OPSCC and 20 oral cavity cancer patients were included. All controls tested HPV-negative. HPV16 was detected in 21/25 OPSCC tumor swabs (84%). Mean HPV16 fold change was 7,503.08 in tumors versus 1.29 in contralateral normal mucosa ($p < 0.0001$). One qPCR-negative swab was HPV33-positive on sequencing. Overall sensitivity was 88% and specificity 100%.

Results: A total of 25 patients with biopsy-proven OPSCC and 20 patients with oral cavity squamous cell carcinoma were included in the study. All oral cavity samples tested negative for HPV. 21 of 25 OPSCC swab samples tested positive for HPV16 on qPCR (84%). The mean fold change of HPV16 was 7,503.08 for tumor swabs compared to 1.29 for contralateral normal oropharyngeal mucosa ($p < 0.0001$). Of the swabs that tested negative for HPV16, one swab sample was positive for MY09/MY11 and was determined to be serotype HPV33 by Sanger sequencing. The overall sensitivity was thus 88% and specificity was 100%.

Conclusions: Here, we validate a novel swab-based detection method of HPV DNA in OPSCC. This method is highly sensitive, specific, minimally invasive, and has potential for tumor characteristic including HPV genotyping that would otherwise only be able to be assessed through labor intensive tissue pathological analysis. This swab-based pathway provides a site-specific scalable diagnostic strategy that holds promise for improving early detection and strengthening surveillance of HPV-associated OPSCC in routine clinical practice.

The Molecular Landscape of Inner Ear Schwannoma

9:45 a.m. - 10:00 a.m.

Jonas Scheffler*¹, Sandra Leisz¹, Eric Lehner¹, Jessica Stieber¹, Elisa Darnstaedt¹, Sabine Koitzsch¹, Tony Gutschner¹, Arne Liebau¹, Stefan K. Plontke¹

¹*University Medicine Halle (Saale)*

Background: Inner Ear Schwannoma (IES) represent a rare subset of vestibulocochlear schwannoma (VS). Their intralabyrinthine location and close proximity to sensitive modiolar structures, including spiral ganglion and sensory hair cells, imply diagnostic, prognostic and therapeutic challenges compared to VS in the cerebellopontine angle (CPA) or internal auditory canal (IAC). Moreover, the microenvironment surrounding IES differs substantially from that of VS adjacent to the brainstem. Although clinical reports and case series on IES are steadily increasing, the tumor biology remains poorly understood. This study aims to provide a comprehensive overview of the key molecular characteristics of IES at both the RNA- and DNA-levels.

Methods: Fresh-frozen tumor samples from 15 patients with IES were collected during surgical resection. Bulk RNA sequencing was performed on all 15 samples, and whole-genome sequencing on 10. A control cohort comprised of published data of VS from CPA/IAC. Bioinformatic analyses included differential gene expression, pathway enrichment, cell-type deconvolution, as well as variant calling, copy-number/ structural variation analysis, and mutational signature profiling. Integrated RNA–DNA analyses were conducted to characterize molecular features of IES in relation to their specific location within the inner ear.

Results: Integrative genomic and transcriptomic analyses identified molecular features of IES. The datasets showed alterations at transcriptional levels, involving pathways relevant to tumor biology, location and the tumor microenvironment.

Conclusions: This study provides the first integrative molecular characterization of IES. These findings advance the understanding of IES biology and provide a foundation for the development of improved diagnostic and therapeutic approaches.

Podium 4: Multi-disciplinary Research of the Middle Ear

8:00 a.m. - 10:00 a.m.

Meeting Room 208ABC, Floor 2

Moderators: Regina Gendzelevski Kelmann and Z. Jason Qian

Impact of Age and Sensorineural Hearing Loss on Sound Transmission Through the Middle Ear

8:00 a.m. - 8:15 a.m.

Stefan Stenfelt*¹

¹*Linköping University*

Background: Sound transmission typically occurs through air conduction (AC): sound waves enter the outer ear, are converted into vibrations by the tympanic membrane, and are transmitted via the ossicles to the inner ear. Bone conduction (BC) provides an alternative pathway, bypassing the middle ear. Traditionally, middle ear sound transmission—assessed through the difference between AC and BC thresholds—has been regarded as stable and unaffected by aging or sensorineural hearing loss (SNHL). However, recent evidence suggests that age and SNHL may influence this relationship. The present study aimed to evaluate how aging and SNHL affect middle ear sound transmission, measured as the AC–BC threshold difference.

Methods: Audiometric data from 13,417 participants (ages 4–102 years, mean 59.3 years) were extracted from the Linköping University Hospital audiology database (2018–2019). Bilateral AC and BC thresholds between 250–4000 Hz were analyzed. Ears with presumed conductive hearing loss, defined as ≥ 25 dB difference between AC and BC at two or more frequencies, were excluded. Effects of age, gender, and SNHL on AC–BC differences were assessed using regression modeling.

Results: Across the cohort, significant AC–BC differences were observed: ~ 5 dB at 250, 1000, and 3000 Hz, and 11 dB at 4000 Hz. Females demonstrated slightly greater differences (~ 1 dB) than males, likely reflecting reference threshold standards. Children and adolescents (< 20 years) exhibited higher AC–BC discrepancies than adults. Regression analysis showed negligible influence of age on AC–BC differences, except at 500 Hz where middle ear transmission improved slightly (~ 0.5 dB per decade). When AC thresholds were used as reference, regression models indicated poorer middle ear transmission at ≤ 1 kHz with greater hearing loss, whereas using BC thresholds suggested the opposite—improved transmission. At higher frequencies, both reference systems indicated worsening transmission with increased loss. However, detailed analysis suggested these findings were largely attributable to measurement constraints, particularly at the extremes of AC and BC testing ranges.

Conclusions: Children exhibited systematically larger AC–BC differences, likely due to developmental or calibration-related factors, producing spurious indications of conductive loss. Small gender-related differences appear to arise from anatomical variation. In adults, middle ear transmission remains stable across age, with no meaningful impact of SNHL. Previously reported effects of aging or SNHL on middle ear sound transmission are most likely artifacts of test limitations, particularly with BC at threshold extremes. Additional contributors to variability may include vibrotactile responses, harmonic distortion, airborne radiation, binaural summation, and calibration differences between AC and BC transducers.

Improved Automatic Segmentation of the Ossicular Chain From Cone-Beam Ct Using Nnu-Net With an Expanded Dataset of 2,000 Scans

8:15 a.m. - 8:30 a.m.

Liam Barrett^{*1}, Talisa Ross², Anne Schilder³, Nishchay Mehta²

¹*University College London*, ²*Royal National Ear Nose and Throat Hospital*, ³*Ear Institute University College London*

Background: Automated segmentation of the ossicular chain from medical imaging is crucial for otological diagnosis, surgical planning, and treatment monitoring. Whilst convolutional neural networks have shown promise in middle ear segmentation, existing studies have been severely limited by dataset size. Our systematic review identified only 866 scans across all published studies, with individual studies typically using fewer than 200 scans. The stapes has proven particularly challenging, with even the best-performing architectures achieving Dice similarity coefficients of only 0.84, compared to over 0.90 for the malleus and incus. This research addresses these limitations by leveraging a substantially larger dataset to determine whether increased training data improves segmentation performance, particularly for challenging anatomical structures.

Methods: We utilised 2,000 cone-beam CT scans from University College London Hospitals, representing a three- to ten-fold increase over previous studies. Between 200 scans were manually annotated by expert clinicians for the complete ossicular chain and individual components (malleus, incus, stapes). The nnU-Net architecture was selected based on its self-configuring capabilities and proven performance in medical image segmentation. We trained three model variants: 3D low-resolution, 3D full-resolution, and 3D cascade, using five-fold cross-validation. Performance was evaluated using Dice similarity coefficient, average symmetric surface distance, modified Hausdorff distance, and 95th percentile Hausdorff distance. We investigated the relationship between training set size and segmentation accuracy through incremental training experiments.

Results: Analysis indicates improved segmentation performance across all ossicular components compared to literature benchmarks. The expanded dataset demonstrates particular benefit for stapes segmentation, historically the most challenging structure. Performance scaling analysis reveals a distinct pattern in the relationship between dataset size and segmentation accuracy across ossicular structures. The malleus and incus achieved state-of-the-art performance after approximately 70 training scans, with Dice coefficients plateauing at 0.92 and 0.93 respectively. In contrast, stapes segmentation continued to improve with increasing dataset size up to 120 scans (Dice=0.88), demonstrating that smaller anatomical structures benefit disproportionately from larger training datasets. Notably, we did not observe a ceiling effect in model capacity; more complex architectures (3D full-resolution and 3D cascade) consistently outperformed simpler models without exhibiting overfitting, suggesting that both data quantity and model complexity contribute to optimal performance. These results demonstrate that the combination of expanded training data and sophisticated architecture selection enables clinically viable automated segmentation across all ossicular components.

Conclusions: This study demonstrates that substantially larger training datasets can overcome historical limitations in automated ossicular chain segmentation, particularly benefiting small anatomical structures like the stapes. Our findings suggest that data scarcity has been a significant constraint in achieving clinically viable segmentation accuracy. These results have immediate implications for clinical workflow integration, potentially enabling automated preoperative planning and quantitative assessment of middle ear pathology. Future work should focus on validating performance across multiple institutions and extending to pathological cases.

Background and Procedure-Related Histologic Findings in Otic Tissues of Four Laboratory Species

8:30 a.m. - 8:45 a.m.

Cory Howard*¹, Rachel Tapp², Mee Ja Sula²

¹Michigan State University Veterinary Diagnostic Lab, ²Charles River Laboratories

Background: Preclinical ototoxicity studies evaluate drug candidates intended for human use and typically include histologic examination of the external, middle, and inner ear, along with auditory brainstem response (ABR) and cytochleogram assessments. Larger mammalian species such as guinea pigs, rats, nonhuman primates, and chinchillas are commonly used as animal models. Despite the importance of these studies for investigational new drug applications to proceed with clinical trials, limited information is available on background and procedure-related histologic findings in control animals. Such information is critical for determining whether observed changes represent test article-related effects.

Methods: Histologic observations were retrospectively collected from ototoxicity studies conducted at Charles River Laboratories (formerly MPI Research; Mattawan, MI). Only control groups (vehicle- or placebo-treated animals) were included. Fifty-one studies met inclusion criteria, encompassing 42 cynomolgus macaques, 437 guinea pigs, 217 Sprague Dawley rats, and 56 cats. Observations were grouped by route of administration: cynomolgus macaques received transtympanic injection through the tympanic membrane with deposition of test article into the tympanic cavity (n = 31) or mastoidotomy and direct administration into the cochlea via the round window membrane or semicircular canals (n = 11); guinea pigs received middle ear catheter (n = 105), transtympanic (n = 268), or systemic (n = 64) administration; rats received systemic (n = 83), transtympanic (n = 128), or topical (n = 6) administration; and cats received only transtympanic administration (n = 56). Histopathologic observations were combined regardless of severity or laterality. Percent incidence was calculated for each observation.

Results: Distinct species-specific patterns were observed. In systemically treated guinea pigs, the most common histologic findings were new bone formation in the ossicles (40.6%) and tympanic bulla (35.9%), while in Sprague Dawley rats, mucoperiosteal mineralization (31.3%) and tympanic cavity infiltration/inflammation (26.5%) were most common. Guinea pigs with indwelling catheters had middle ear new bone formation (7.6%), as well as extensive fibroplasia (45.7%) and inflammation (35.2%) centered on the catheter insertion site. In cynomolgus macaques, tympanic cavity new bone formation and fibroplasia were seen in all animals (100%) undergoing mastoidotomy and intracochlear administration while only a few animals (6.5%) undergoing transtympanic administration had new bone formation. Mastoidotomy was also associated with hemorrhage (45.5%), foreign material (90.9%) and inflammation (54.5%) in the tympanic cavity. Tympanic membrane fibrosis was common in both mastoidotomy (81.8%) and transtympanic (77.4%) administration. Across all species and routes of administration, pigmented/foamy macrophages in the tympanic cavity was common.

Conclusions: Species and route of administration affect the background and procedure-related incidence of histologic findings in preclinical ototoxicity studies. These observations must be considered when determining whether findings represent test article-related effects.

The Effects of Basic Fibroblast Growth Factor and Gelatin Sponge Administration on Blast-Induced Tympanic Membrane Perforation in Mice

8:45 a.m. - 9:00 a.m.

Shingo Yasutake^{*1}, Kunio Mizutani², Takaomi Kurioka³, Yasushi Satoh¹

¹National Defense Medical College, ²Tokyo Women's Medical University Adachi Medical Center, ³Kitasato University School of Medicine

Background: In recent conflicts and terrorism, blast injuries caused by bombs and explosives are a common possibility. Although tympanic membrane perforation (TMP) is known to be the most frequent auditory complication of blast injuries, its healing rate is generally poor. While basic fibroblast growth factor (bFGF)-containing gelatin sponges (gel) have been shown to be effective in the treatment of TMP, studies on their use for blast-induced TMP haven't been conducted. Using and developing a blast injury mouse model, we have previously researched inner ear and middle ear injuries. In the present study, we investigated the effects of bFGF and gelatin sponge administration on blast-induced TMP in mice.

Methods: Male CBA/J mice, 10-12 weeks of age, were divided into five groups: (1) control (non-blast-exposed), (2) no treatment (untreated blast-exposed TMP ears), (3) bFGF (blast-exposed TMP ears treated with bFGF eardrop), (4) gel (blast-exposed TMP ears treated with gel placement into the TMP), and (5) bFGF + gel (blast-exposed TMP ears treated with bFGF immersed gel placement into the TMP). The blast-tube system generated a peak pressure of 330 ± 11.3 kPa from the blast shock wave and irradiated the mice from the front of their heads. TMP size was measured over time, auditory function was assessed using auditory brainstem response (ABR) and distortion product otoacoustic emissions (DPOAEs), and cochlear damage was evaluated via hair cell survival. Two months after the blast exposure, the fixed middle ear of mice embedded in paraffin, and the tympanic membrane (TM) was histologically evaluated for thickness.

Results: Combination treatment with bFGF and gel promoted better TMP healing, with thinner, structurally improved tympanic membranes compared to other groups. There were no effects of drug administrations, such as bFGF or gel, on ABR, DPOAE and hair cell survival rates.

Conclusions: These findings suggest that bFGF with a gelatin scaffold may effectively enhance TMP repair following blast injuries. This study provides new insights into potential therapeutic strategies for blast-related auditory injuries, which we will discuss alongside detailed auditory and histological assessments.

Osteoclasts Begin to Migrate From the Perivascular Area in Lps Induced Otitis Media Mouse Model

9:00 a.m. - 9:15 a.m.

Sho Kanzaki^{*1}, Yukiko Kuroda², Koichi Matsuo²

¹National Institute of Sensory Organ Tokyo Medical Center, ²Laboratory of Cell and Tissue Biology, Keio University School of Medicine, Tokyo, Japan

Background: Recurrent or chronic otitis media has long been associated with hearing loss, yet the mechanisms that underlie this relationship remain incompletely defined. One possible explanation is that hearing loss arises as a consequence of inflammation-induced activation of osteoclasts, the specialized bone-resorbing cells. Inflammatory conditions create an acidic microenvironment that weakens the bone matrix, and osteoclasts accelerate this degradation, leading to histologically distinct resorption. Repeated or chronic episodes of otitis media are more likely to result in progressive bone destruction. However, it is not yet clear whether acute otitis media itself is sufficient to mobilize osteoclasts and initiate ossicular bone damage, or from which cellular niche these osteoclasts arise. Clarifying the dynamics of osteoclast recruitment in the setting of otitis media is therefore essential to understanding disease-related bone pathology and its link to hearing loss.

Methods: To investigate this question, we established an experimental model of otitis media in mice. Lipopolysaccharide (LPS), a component of Gram-negative bacterial cell walls known to elicit strong inflammatory responses, was injected into the middle ear cavity to induce otitis media. As a control, phosphate-buffered saline (PBS) was injected into the middle ear of separate mice. Ossicles were harvested for histological and imaging analyses. Osteoclast localization was assessed using tissue sections, and three-dimensional visualization was achieved with light-sheet microscopy on optically cleared ossicular samples. This approach allowed us to evaluate not only the number of osteoclasts but also their spatial relationship to surrounding vascular structures.

Results: Our analyses revealed the presence of osteoclasts in perivascular regions of the ossicles. A higher number of osteoclasts was observed in LPS-treated mice compared to PBS controls, with the malleus demonstrating the most pronounced difference. These findings indicate that osteoclast activity is upregulated in response to LPS-induced middle ear inflammation. Importantly, the perivascular positioning of these osteoclasts suggests that blood vessels act as a key site for their recruitment and activation,

Conclusions: Osteoclasts were consistently found in perivascular regions within the ossicles of LPS-inoculated mice. The perivascular localization of osteoclasts implies that osteoclasts may originate from circulating precursors and subsequently contribute to bone resorption from within the ossicular chain. This osteoclastic activity may create focal porosity in the ossicles during otitis media, thereby weakening their structural integrity. Our findings highlight a potential mechanism by which inflammatory processes in otitis media drive osteoclast formation and subsequent ossicular bone resorption. This insight not only advances understanding of how chronic infections lead to hearing loss but also points to possible preventive strategies that target osteoclast recruitment or function, with the ultimate goal of mitigating disease-related auditory impairment.

Regulation of Middle Ear Genes During Otitis Media by Changes in Dna Accessibility

9:15 a.m. - 9:30 a.m.

Allen F. Ryan^{*1}, Nathan Zemke¹, Arwa Kurabi¹

¹*University of California, San Diego*

Background: The middle ear (ME) mounts a three-pronged initial defense when invaded by bacteria: mucus, innate immune receptors, and effectors including cytokines, chemokines and

antimicrobial peptides which are mobilized when innate immune receptors are activated. While gene expression studies document these defense processes during otitis media (OM), how they are regulated is less well known.

Methods: Single-cell RNA-Seq was used to assess expression of genes, including regulatory transcription factors (TFs), throughout an episode of acute OM in the mouse. Single-nuclear ATAC was employed to document changes in defense gene DNA accessibility in the neighborhood of their transcribed regions. Transcription factor binding sites within altered DNA regions were mapped, and compared to TF expression.

Results: OM-associated changes in DNA accessibility were identified for many defense genes whose expression was also altered, exposing binding sites for TFs. For other defense genes, accessibility changes were minimal. For a few genes, some accessible regions were eliminated by infection. TFs associated with binding sites in accessible regions that showed significant changes during OM included HIF1A.

Conclusions: Changes in DNA accessibility appear to be involved in the regulation of many genes expressed by ME cells during otitis media. The presence of HIF1A binding sites, coupled with increased expression during OM, suggests a role for hypoxia in middle ear gene regulation. For genes in which changes in DNA accessibility were minimal or absent, altered expression may be regulated by changes in TF expression, DNA methylation, and/or alterations in DNA accessibility at distant sites.

Rare and Low-Frequency Genetic Variants in Families With Otitis Media

9:30 a.m. - 9:45 a.m.

Regie Lyn Santos-Cortez¹, Christina Elling¹, Helen Gomez¹, Elisabet Einarsdottir², Juha Kere², Petri Mattila², Lena Hafrén², Allen Ryan³

¹University of Colorado Anschutz Medical Campus, ²University of Helsinki, ³University of California San Diego

Background: Otitis media is a highly frequent diagnosis in children that causes significant morbidity but remains understudied as a genetic trait despite significant heritability in families. Our goal was to identify rare or low-frequency variants within genes that confer susceptibility to otitis media.

Methods: Exome sequence data of 287 individuals from 243 families were analyzed. Identified variants were tested for co-segregation with otitis media in family members. Genome sequence data from a case-control cohort was imputed and analyzed for association of specific genes with otitis media. Single-cell RNA-sequence data of identified genes were noted in acutely infected mouse middle ears.

Results: Thirty-three variants within 24 genes co-segregated with otitis media in 28 families, of which 18 variants were considered pathogenic or likely pathogenic. An additional 81 variants in 21 of the same genes were identified in 83 unrelated probands with otitis media. Of the 24 genes, twelve were associated with otitis media in mouse models, while fifteen genes were replicated from previous human studies. A common variant EYA4 c.829G > A was associated with otitis media in the case-control cohort. Using network analysis, twenty-two of the 24 genes were connected in a subnetwork enriched in various signaling pathways, Th1/Th2/Th17 cell

differentiation and viral infections. Majority (87.5%) of the identified genes were expressed in mouse middle ear cells, with differential expression after acute infection.

Conclusions: The identification of novel genes and variants for susceptibility to otitis media will be useful in future risk screening and clinical management in children that require a more personalized approach due to poor response to standard treatments.

Unexplained Conductive Hearing Loss in Down Syndrome: Evidence From Audiometric and Imaging Data

9:45 a.m. - 10:00 a.m.

Gabrielle Merchant^{*1}, Heather Porter¹, Kristen Janky¹, Keelin Fallon², Aaron Remenschneider³

¹Boys Town National Research Hospital, ²University of Massachusetts Chan Medical School,

³Boston Children's Hospital; Massachusetts Eye and Ear Infirmary

Background: Conductive hearing loss (CHL) affects 50-80% of individuals with Down syndrome (DS), typically attributed to chronic middle ear disease such as otitis media. However, conventional treatments like placement of pressure equalization tubes often fail to resolve CHL, and clinical observations reveal that many individuals with DS demonstrate persistent air-bone gaps without signs of active middle ear pathology. We define this audiometric pattern as unexplained conductive hearing loss (uCHL). While recognized anecdotally, uCHL remains poorly quantified and it is underrepresented in the literature. Notably, inner ear anomalies have been reported in up to 75% of individuals with DS, such as enlarged vestibular aqueducts, superior semicircular canal dehiscence, and cochlear hypoplasia. This study aims to quantify uCHL and explore its association with structural anomalies of the auditory system using audiometric and imaging data.

Methods: We analyzed two preliminary datasets:

1. Retrospective cohort: 46 ears from individuals with DS without active middle ear disease who underwent both audiologic evaluation and temporal bone computed tomography (CT) imaging within a 6-month window (Aug 2023–Aug 2024). CT scans were reviewed for anomalies of the outer, middle, and inner ear.
2. Prospective cohort: 92 ears from 46 individuals with DS, recruited regardless of hearing status, who completed behavioral audiometry, bilateral masked bone conduction testing, tympanometry, and wideband tympanometry (WBT).

Results: In the retrospective cohort, all 46 ears exhibited air-bone gaps (29 ears had CHL and 17 had mixed hearing loss), yet 70% had normal tympanometry, consistent with uCHL. CT findings showed inner ear anomalies in 91% of ears, middle ear anomalies in 83%, and outer ear anomalies in 70%, challenging the assumption that CHL in DS arises solely from middle ear dysfunction. Additional associations between imaging and audiometric data will be presented.

In the prospective cohort, 63% of ears (58/92) demonstrated air-bone gaps, and 76% of those (44/58) had normal tympanometry. WBT confirmed normal middle ear absorbance in 64% (37/58) using conservative normative criteria. Notably, masked bone conduction thresholds were frequently seen in the negative dB HL range in conjunction with air-bone gaps, suggesting

hypersensitive bone conduction. These findings were absent in neurotypical controls using identical equipment and protocols, ruling out calibration artifacts.

Conclusions: uCHL is prevalent in DS and often co-occurs with normal middle ear function on standard assessments, indicating that conventional diagnostic models may be insufficient. The high prevalence of inner ear anomalies and enhanced bone conduction thresholds suggests multifactorial or novel mechanisms, such as third window effects or altered bone conduction dynamics. These findings highlight the need for expanded diagnostic approaches in DS and further research into the pathophysiology and mechanism of uCHL in this population.

Symposium 1: In Vitro Models as Relevant Tools to Support Translational Hearing Research

8:00 a.m. - 10:00 a.m.

Meeting Room 209ABC, Floor 2

In Vitro Models as Relevant Tools to Support Translational Hearing Research

Natalia Smith-Cortinez, *University Medical Center Utrecht*

Symposium Description: Despite decades of dedicated research, hearing loss remains without a single FDA-approved therapeutic for its treatment—a stark reflection of the translational challenges facing the field. A key barrier to progress is the limited predictive power of traditional animal models and the scarcity of physiologically relevant in vitro systems that can bridge the gap between discovery and clinical application. This symposium will spotlight the need for a critical shift in regulatory and scientific strategy. We will highlight emerging high-throughput platforms and human centered strategies designed to accelerate therapeutic discovery. This include cochlear and inner ear organoids derived from patient-specific induced pluripotent stem cells (iPSCs), which offer new opportunities to model genetic forms of deafness. We will also discuss the potential of patient- or cadaver-derived cochlear stem cells for drug screening and precision medicine. This encompasses traditional models such as explants to human based organoids A particular focus will be given to recent advances in the in vitro expansion of auditory progenitor cells, which enable regenerative strategies and improve experimental scalability. By bringing together diverse voices from across auditory neuroscience, bioengineering, and the clinic, the symposium aims to catalyze interdisciplinary dialogue, inspire innovative collaborations, and build new bridges within the hearing research community. From a translational perspective, we will examine how these in vitro approaches can complement and enhance existing clinical standards, including cochlear implants, and how researchers might improve their efficacy and integration. Together, these platforms form a comprehensive translational framework for inner ear research, bridging basic science and clinical application and driving the development of next-generation therapeutics for hearing loss.

Erik de Vrieze, *Dept. of Otorhinolaryngology, Radboud University Medical Center, Nijmegen, The Netherlands*

Monica Serban, *University of Montana*

Francis Rousset, *University of Geneva*

Human Cellular Models of the Inner Ear

Konstantina Stankovic, *Stanford School of Medicine*

Individual Abstract: Hearing loss represents a significant global health challenge, reaching pandemic levels. The most prevalent form of this condition is sensorineural hearing loss (SNHL), which typically arises from damage to the inner ear's cellular structures. Human induced pluripotent stem cells (hiPSCs) can be derived from individuals, including those affected by various types of SNHL. Advancements in differentiation protocols aimed at producing inner ear cells, such as hair cells (HCs) and spiral ganglion neurons (SGNs), hold great promise for enhancing and understanding of human ear disease, evaluating potential treatments, and developing cell-based therapies. We demonstrate how hiPSCs-derived otic progenitor cells, Schwann cells, and organoids offer fresh perspective on virally-induced SNHL, including those lined to cytomegalovirus, Zika virus, and SARS-CoV-2. Additionally, we highlight how these innovative cellular models of the inner ear facilitate research into the impact of oxidative stress on the structure and function of inner ear tissues.

Human Inner Ear Organoids as a Versatile Platform to Model and Treat Acquired and Genetic Hearing Loss

Heiko Locher, *Leiden University Medical Center*

Individual Abstract: Human inner ear organoids derived from induced pluripotent stem cells (hiPSCs) are emerging as a powerful in vitro platform to study sensorineural hearing loss. These 3D models recapitulate key aspects of human inner ear development and cellular complexity, enabling investigation of both genetic and acquired causes of hearing loss. We employed inner ear organoids to model monogenic disorders, assess antisense oligonucleotide (ASO) therapies, and to study environmental insults such as ototoxic drug exposure and congenital cytomegalovirus (CMV) infection. To include relevant genotypes, inner ear organoids were generated from healthy, patient-derived, and isogenic mutant hiPSC lines. For genetic modeling, we focused on mutations in *USH2A* and *COCH*, applying ASOs via gymnotic delivery to modulate RNA expression. Organoids from healthy hiPSC lines were also exposed to known ototoxic agents (cisplatin, gentamicin) and CMV laboratory and clinical strains to assess damage and cell-type-specific vulnerability. Disease and treatment effects were evaluated using immunohistochemistry, RNAscope, qPCR, and confocal microscopy. All organoids exhibited hallmark features of the inner ear, including stereociliated hair cells and periotic mesenchyme. Patient-specific and gene-edited lines recapitulated expression of mutant transcripts and disease phenotypes. ASO treatment led to significant reductions in mutant RNA levels and partial rescue of molecular defects. Additionally, ototoxic agents and CMV infection induced selective damage to hair cells and neuronal structures, demonstrating the model's utility for studying acquired hearing loss. Our work highlights the value of using human inner ear organoids as a translational platform to dissect the molecular mechanisms underlying genetic and acquired forms of hearing loss. They enable therapeutic testing across a range of etiologies, from RNA-targeted therapies to

infection and drug-induced damage, paving the way for more personalized and predictive treatment strategies.

Progenitor Cells in the Adult Human Express Lgr5 and Have the Potential to Produce MYO7A+ Cells in Vitro

Louise Straatman, *University Medical Center Utrecht*

Individual Abstract: In the embryonic cochlea, supporting cells give rise to sensory hair cells. In adult mice, supporting cells retain expression of progenitor markers such as Lgr5 and Sox2, yet spontaneous hair cell regeneration does not occur. Most previous studies have focused on neonatal mouse cochlear explants, which limits translational relevance due to species and developmental differences, as hearing loss primarily affects adults. For translational purposes this study assessed the regenerative potential of the adult human inner ear.

We evaluated LGR5 expression in adult human inner ear tissue obtained within 12 hours postmortem from bodies donated to science. We also assessed the progenitor potential of inner ear sensory epithelium from adult patients undergoing skull-base surgery, and from young adult and aged Lgr5-GFP mice with normal hearing or hearing loss. Cells were cultured in 3D and key signaling pathways were manipulated to induce proliferation and differentiation of the cells.

Our findings show that LGR5 is expressed in the adult human cochlea and vestibular organ. Supporting cells from sensory epithelium from human inner ear and adult mouse cochlea demonstrated the capacity to proliferate and form organoids in vitro. After differentiation, MYO7A+

hair cell-like cells were generated in these organoids.

These results indicate that progenitor cells with regenerative potential are present in the human and mouse adult cochlea and vestibular organ. When activated through targeted manipulation of signaling pathways, these cells can proliferate and give rise to hair cell-like cells. This highlights their promise as therapeutic target cells for future therapies to restore hearing in adults.

A Scalable in Vitro Platform for High-Throughput Drug Development in Primary Auditory Neurons

Francis Rousset, *University of Geneva*

Individual Abstract: Sensorineural hearing loss remains an incurable condition, primarily due to

the irreversible loss of cochlear hair cells and auditory neurons. A major barrier to therapeutic development is the lack of in vitro models that faithfully recapitulate auditory neurobiology while

supporting high-throughput screening (HTS). Although rodent explants and in vivo models have provided valuable insights, their limited throughput and logistical constraints limit their utility in early-stage drug discovery, where screening large compound libraries is essential.

We have recently established a robust in vitro protocol to expand auditory neuroprogenitor cells

(ANPGs), enabling the scalable production of auditory neuron-like cells. By simultaneously activating WNT signaling and inhibiting the TGF β /Smad pathway, quiescent ANPGs are reprogrammed into a highly proliferative state. These so-called Phoenix cells combine sustained self-renewal with robust neuronal differentiation capacity, offering a stable, primary-like model system. Furthermore, they can be frozen and thawed, eliminating the need for continuous animal tissue supply while ensuring reproducible, batch-consistent assays.

Building on this foundation, we developed a high-throughput platform to screen large libraries of compounds that promote auditory neuron protection or regeneration. To enable real-time monitoring of auditory neurogenesis and neuroprotection, we implemented a live-cell genetic reporter system in 384-well plate assays. This automated system supports both phenotypic and genetic screening approaches, facilitating the discovery of novel genetic targets and candidate compounds.

Altogether, the Phoenix platform offers a scalable, reproducible, and physiologically relevant alternative to animal-based models, accelerating early-stage drug discovery and advancing the preclinical pipeline for inner ear therapeutics.

Building Your Own Toolbox: A Journey Developing and Utilizing in Vitro 3D Tissue Models to Enable High-Throughput Screening of Novel Othotherapeutics and Drug Delivery Devices

Joachim Veit, *University of Montana, Missoula*

Individual Abstract: Despite otic disorders affecting a substantial portion of the global population, othotherapeutics, particularly those intended to treat hearing loss, have seen limited development or innovation. This is in part due to the limited tool set available to the field which consequently relies heavily on costly and low throughput preclinical animal models with often ambiguous human relevance. This presentation highlights our journey in developing in vitro tympanic and round window membrane 3D human tissue models to enable high-throughput screening of topically applied othotherapeutics. These models were subsequently employed in the characterization of several novel topical therapeutics targeting the oxidation- and inflammation-mediated damage to inner ear cells which is concurrent in nearly all insults causing hearing loss. Finally, these in vitro models were utilized to prototype a series of microneedle-based drug delivery devices with tailorable tympanic residence times. These devices facilitate the transtympanic permeation of topically applied othotherapeutics to allow for recurring therapeutic access to the middle and inner ear. Together, this project highlights the value of targeted efforts to develop and make use of novel in vitro research tools which can provide lower cost, higher throughput, and increased human relevance options over preclinical animal models for translational otic research and beyond.

Cochlear Explant as a Reference Ex Vivo Model and a Platform for Developing in Vitro Systems

Marianne Aincy, *Cilcare*

Individual Abstract: Cochlear explant cultures offer a valuable and reliable approach for studying drug-induced ototoxicity and potential otoprotective effects, although validation through in vivo models remains necessary. This model provides a versatile, ethical framework suitable for multiple applications: dose-response assessments of ototoxic compounds, exploration of their mechanisms of action, and efficacy evaluation of protective treatments. It also serves as reference for developing human in vitro models such as organoids derived from hiPSCs.

To facilitate screening for otoprotective drugs while adhering to 3Rs principles (Replacement, Reduction, Refinement), we developed and optimized a robust ex vivo cochlear explant model. This model employs cochlear explants exposed to cisplatin and aminoglycosides, widely used chemotherapeutic and antibiotic agents known to cause irreversible cochlear hair cell damage and permanent hearing loss.

Our model uses neonatal rodent pups, significantly reducing animal usage by minimizing gestating females required. It offers advantages, including avoidance of systemic administration and associated ethical issues. It provides precise control over experimental conditions, ensuring consistent, direct exposure of cochlear cells to dosed treatments. The model is efficient, with standardized culture limited to 96 hours.

However, technical challenges exist. Successful explant preparation and maintenance require specialized expertise in dissection, culture media selection, and aseptic techniques. The limited lifespan of explants restricts their use in long-term studies. Additionally, the developmental immaturity of cochleae from pups aged P1-P4 may limit translational applicability to mature auditory systems. Importantly, explant models complement but do not fully replicate systemic complexity found in living organisms.

Our goal was to refine this model by addressing critical technical parameters: choice of rodent species (mouse vs. rat), pup age, dissection techniques, culture medium composition, and exposure specifics for ototoxic agents (cisplatin, kanamycin, gentamicin, tobramycin). Ototoxic severity can be adjusted by modifying these parameters to selectively target particular cochlear structures.

We validated multiple cellular markers to visualize and quantify essential cochlear components: hair cells, supporting cells, nerve fibers, ribbon synapses, and neurons. Advanced laser scanning confocal microscopy and histological analyses were employed for comprehensive qualitative and quantitative assessments.

Our cochlear explant model provides a powerful, flexible method for studying ototoxicity and identifying promising otoprotective agents, ultimately reducing reliance on in vivo testing. Building on this robust reference system, our ongoing efforts focus on developing complementary human in vitro models, including inner ear organoids and 3D-printed round window platform. Together, these models bridge fundamental research and translational applications, paving the way for innovative therapeutic approaches to hearing preservation.

Why Don't Cochlear Implants Work Better?

Manohar Bance, *University of Cambridge*

Individual Abstract: The cochlear implant-neural interface is difficult to interrogate in living CI users, and in animals the interface can differ significantly because of the size and shape of the cochlea, and different implants. CI users present with varying symptoms and complaints, and to back project to the underlying cause requires some methods to understand what is happening at the CI-neural interface. We have used a variety of models to try to understand how changes at this interface might manifest in terms of hearing performance, cross stimulation of other structures, or insertion trauma. The types of models I will discuss are: 1) Computational models which try to understand how nerves are activated by CIs, and the physical spread of current in the cochlea. 2) Physical 3D printed which help us understand insertion trauma forces, and electric field distribution inside the cochlea. 3) Cadaveric models which allow us to make measurements in realistic human like geometries. 4) Cellular models which let us measure responses of neurones to different types of stimulation, and also to simulate scarring and fibrosis. I will discuss the lessons learned from these various models, and the clinical applications of what we have learnt.

Podium 5: Auditory Prostheses #1

10:30 a.m. - 12:30 p.m.

Ballroom B, Floor 3

Cortical Encodings of Acoustic and Electrical Cochlear Stimulation Are Distinct

10:30 a.m. - 10:45 a.m.

Ariel Hight^{*1}, Michele Insanally², Julia K. Scarpa³, Yew Song Cheng⁴, Michael Trumpis⁵, Jonathan Viventi⁵, Mario Svirsky¹, Robert Froemke¹

¹New York University Grossman School of Medicine, ²University of Pittsburgh School of Medicine, ³Weil Cornell New York Presbyterian Hospital, ⁴UCSF, ⁵Duke University School of Medicine

Background: Cochlear implants are neuroprosthetic devices that restore hearing to folks with severe to profound hearing loss, providing abilities to attain open-set speech perception without aids of other sensory cues such as lip reading. Despite widespread adoption, there is limited understanding of how these devices restore functionality and the sense of hearing. One long-standing question has been how central auditory pathways are activated by stimulation by cochlear implants and to which degree this activation overlaps with activation evoked by similar acoustic stimuli under normal hearing conditions. In this study, we determined the spatial and temporal representations of both tones and cochlear implant channels across the broad extent of the auditory cortex (A1) by using micro-electrocorticography (μ ECoG), a subtype of intracranial electroencephalography (iEEG).

Methods: Stimulus evoked responses were acquired from 60 cortical surface electrodes (3.25 x 3.25 mm grid) placed over the auditory cortex. Evoked responses were analyzed using either event related potentials (ERPs, 2-100 Hz) or high gamma (HG, 70-140 Hz) signals. Tone evoked

responses (1.4-32 kHz) were measured and analyzed from N=7 normal hearing (NH) rats and cochlear-implant evoked responses were recorded and analyzed from N=7 bilaterally deafened rats unilaterally implanted with an 8-channel intracochlear array (CI). Of these rats, 4 underwent both tone-evoked and implant-evoked recordings. We trained classifiers to determine whether we could successfully decode stimulus identity from single trial iEEG measurements for both NH and CI rats-- first we reduced evoked responses using either principal component analysis (PCA) or tensor component analysis (TCA), then trained a linear discriminant analysis (LDA) decoder on 13 randomized trials and then computed prediction probabilities for the remaining trials.

Results: Both tones (NH) and intracochlear electrodes (CI) evoked clear single-trial and trial-averaged transients, and we found that A1 encoding of these responses were topographically organized for both NH and CI stimuli. Next, we found that single-trial evoked responses were more variable for CI vs NH rats, but only in the spatial and not the temporal domain. Then, we trained and tested classifiers on single trials of NH and CI evoked responses and found that stimulus identity could be correctly predicted for both NH and CI evoked responses. Lastly, for the N=4 animals with both NH and CI recordings, we trained a classifier on tone-evoked responses, tested these classifiers on intracochlear-evoked responses, and found minimal information transfer between responses evoked by both modalities.

Conclusions: Our findings provide evidence that acoustic and electrical cochlear implant stimulation evoke fundamentally distinct patterns of cortical activity. The lack of transferable representations suggest that cochlear implant users may experience qualitatively different precepts that cannot be entirely predicted by acoustic hearing. Our results also suggest that refinement of cortical responses may improve inter-neuronal variance at the population level and improve auditory perception.

Why Ramped Pulses Are Reliable Solutions for Coding Sound Intensity in Cochlear Implants : An Electrophysiological Study in the Guinea Pig Auditory Cortex

10:45 a.m. - 11:00 a.m.

Elie Partouche¹, Victor Adenis^{*2}, Pierre Stahl³, Chloe Huetz⁴, Jean-Marc Edeline⁴

¹Paris-Saclay Institut of Neurosciences, ²Mass Eye and Ear, Harvard Medical School, ³Oticon Medical/ Neurelec, ⁴NeuroPSI UMR CNRS 9197

Background: For decades, cochlear implant (CI) is the most successful neuro-prosthetic device allowing more than a million of patients to recover hearing sensation and speech understanding. The performance is usually good in silence, but CI patients have difficulties in the presence of background noise. Potentially, these limitations stem from the large spread of currents diffusing in the cochlea's perilymph when the different electrodes are activated at high current levels. To reduce the spread of current, one potential strategy is to change the shape of the electrical pulses. In human, studies have tested asymmetric rectangular pulses (Macherey et al., 2006, 2008). A new pulse shape, called "ramped pulse", has been proposed (Ballesterio et al., 2015) but only two studies have showed that such pulses elicited responses with lower thresholds than rectangular pulses (Navntoft et al 2020, Partouche et al 2023). Here, we report the consequences of using different types of ramped pulses on responses of auditory cortex (ACx) neurons to increasing electrical charges in anesthetized guinea pigs.

Methods: The intracochlear stimulating array was a shortened version of the EVO electrode array used by Oticon Medical and was composed of 6 ring-shaped Platinum-Iridium electrodes with a 0.0046mm² surface. Four shapes of ramped pulses were tested, each at 20 charge levels (from 3 to 31.5nC), to obtain the growth functions of ACx neurons. Recordings in ACx were collected by 16-channels electrodes composed of two rows of 8 electrodes. Two types of doubles ramped pulses were tested: one with the ramps on the 1st and 4th phases, a second with the ramps on the 1st and 3rd phases. We also tested single ramp pulses with the ramp on the 3rd phase

Results: Compared with the responses obtained with rectangular pulses, all ramped pulses generated (i) responses with lower thresholds (ii) higher maximal firing rates (FR) (iii) higher FR at threshold (iv) longer response latencies and (v) longer durations. These effects were always significant with cathodic-first phase, and were in the same direction (but less often significant) with anodic-first phase. The responses to increasing levels of charges were also compared with the responses to pure tones presented at eight intensities (from 5 to 75dB SPL).

Conclusions: These results indicate that ramped pulses should be considered as a good alternative to rectangular pulses, even if polarity of the first phase matters to benefit from this shape.

Comparison of Two Subjective Tests for Cochlear Implant Users Using Reverberant Speech Processed With an Established Dereverberation Algorithm

11:00 a.m. - 11:15 a.m.

Shaliza Merchant^{*1}, Christiaan Stronks¹, Nienke Langerak¹, Daphne Geijssen¹, Esther Van Marrewijk¹, Jeroen Briaire¹, Johan Frijns¹

¹*Leiden University Medical Center*

Background: Cochlear implants (CI) effectively restore speech perception in quiet conditions for individuals with severe-to-profound sensorineural hearing loss. However, speech intelligibility (SI) in background noise is severely impaired and reverberant environments are challenging for CI users. To mitigate the impact of background noise and reverberation, a variety of speech enhancement strategies have been developed. Performance is often assessed in terms of their effects on SI. However, it is known that algorithms not affecting SI may still be perceived as helpful, while algorithms that effectively enhance SI may be disliked because of speech distortion, underscoring the need for subjective assessment. Here, we compare two subjective tests using reverberant speech processed with an established dereverberation algorithm. The first test was the gold standard in subjectively perceived sound quality: the Multiple Stimulus with Hidden Reference and Anchor (MuSHRA) method. This method was compared with a simplified version, dubbed ‘MuSHRA-lite’.

Methods: Two cohorts of eighteen post-lingually deafened CI users participated. One cohort performed the MuSHRA, in which processed speech stimuli were subjectively compared to one common reference. Participants rated each stimulus on a scale from 0 - 100, for three outcome measures: SI, naturalness and listening effort, relative to an openly labeled reference of unprocessed speech and a hidden anchor. The other cohort performed the MuSHRA-lite, which

consisted of pairwise comparisons of processed speech producing preference scores for the same outcome measures. RM-ANOVA and linear mixed modeling were used to test for statistical significance between the ratings of clean speech, and reverberant and de-reverberated speech.

Results: Both methods confirmed that reverberation significantly degraded SI, naturalness, and listening effort relative to clean speech. However, the perceived benefits of the algorithm differed substantially between methods. MuSHRA-lite consistently revealed statistically significant benefits of the algorithm on all outcomes measures. By contrast, MuSHRA failed to reveal significant effects of the algorithm on any of the outcomes. This lack of statistical power could be coupled to greater variance in the data. Qualitative feedback indicated that performing the MuSHRA was experienced as challenging for many participants, the MuSHRA rating scales were also described as being “difficult” and the task overall as “confusing” (n=4/18).

Conclusions: Both methods captured the detrimental impact of reverberation, but only MuSHRA-lite revealed statistically significant benefits of the dereverberation algorithm on perceived SI, naturalness, and listening effort. Furthermore, MuSHRA-lite was less complicated to implement, easier to execute and interpret. MuSHRA-lite reflects preference between stimuli, while MuSHRA yields quality ratings, which may explain why MuSHRA results were more conservative. We conclude that MuSHRA-lite offers a practical and robust alternative for assessing subjectively perceived speech quality for CI users, especially when preference-based judgements are sufficient and participant burden must be minimized without sacrificing reliability.

Strength–Duration Analysis of Optical and Electrical Auditory Nerve Stimulation Across Species

11:15 a.m. - 11:30 a.m.

Yinchou Zhou¹, Jennifer Silva-Nash¹, Joaquin Cury¹, Xiaodong Tan¹, Claus-Peter Richter¹

¹*Feinberg School of Medicine, Northwestern University*

Background: Cochlear implants (CIs) restore hearing through electrical stimulation of spiral ganglion neurons, yet performance remains constrained by limited frequency resolution and spatial selectivity due to current spread. Optical approaches, including optogenetics (OG) and infrared neural stimulation (INS), promise greater spatial selectivity but require higher energy. While thresholds for these modalities have been reported, a systematic comparison using classic excitability metrics—chronaxie and rheobase—has not been established. Such analyses provide a unified framework for evaluating efficiency, safety, and translational potential across modalities and species.

Methods: We recorded auditory brainstem responses (ABRs) in transgenic mice carrying the optogenetic tool ChR2. We applied light at 460 nm (OG) and at 1375–1550 nm (INS) with a 400 μ m optical fibers at the round window. Electrical stimulation was performed through round window electrodes. For each modality, pulse duration (10–1000 μ s) and radiant exposure/current were systematically varied. Strength–duration curves were fit with the Weiss–Lapicque equation to extract chronaxie and rheobase.

Results: Electrical stimulation showed short chronaxies (~100–200 μ s) with rheobase energies in the sub- μ J range (0.01–0.05 μ J/pulse), confirming efficiency. OG stimulation in mice

exhibited intermediate chronaxies (~300–400 μ s) and rheobase values of ~4–6 μ J/pulse, reflecting selective but energy-intensive activation. INS required the highest energies (10–15 μ J/pulse) with chronaxies > 500 μ s. Across modalities, extending pulse duration beyond the chronaxie failed to further reduce threshold, leading to unnecessary energy expenditure.

Conclusions: By placing electrical, OG, and INS stimulation into a single strength–duration framework, this study identifies fundamental efficiency gaps and defines the optimal temporal regimes for each modality. Cross-species analysis highlights how cochlear anatomy shapes chronaxie/rheobase, underscoring the translational value of guinea pig models. Importantly, the findings support hybrid coding strategies: electrical pulses delivered at threshold can prime neurons, thereby reducing optical thresholds and minimizing thermal load while preserving spatial selectivity. This work provides a physiological basis for designing energy-efficient, opto-electrical cochlear implants aimed at improving pitch perception and spectral resolution in human users.

Effect of Electrode Array Type and Surgical Approach on Structure Preservation Rates During Cochlear Implantation: A Prospective Randomized Controlled Trial (CIPRES Study)

11:30 a.m. - 11:45 a.m.

Imogen van Beurden*¹, Saad Jwair¹, Robert J. Stokroos¹, Huib Versnel¹, Hans G.X.M. Thomeer¹

¹*University Medical Center Utrecht*

Background: Preservation of residual low-frequency hearing during and after cochlear implantation contributes to improving hearing with a cochlear implant (CI), especially in noisy environments. Scalar translocation (STL) is a major threat to hearing preservation during cochlear implantation surgery. Selection of the optimal combination of electrode array and surgical approach could add to better preservation of residual hearing and thus improved hearing with a CI.

Methods: This study is still ongoing. The last participant will be included in September 2025. Forty-seven of 48 adult patients with severe to profound sensorineural hearing loss (SNHL) receiving a CI in a tertiary referral center (UMC Utrecht) were included between January 2020 and September 2025 in an ongoing prospective randomized controlled trial (RCT) comparing surgical approaches (round window or cochleostomy insertion) and electrode arrays models (lateral wall SlimJ array (LW) or perimodiolar MidScala (PM) array, by Advanced Bionics). Participants were randomly assigned to one of four study groups, with 12 participants each: group 1 RW + LW array; group 2 CO + LW; group 3 RW + PM array (Jwair et al., 2021, *Trials* 22:895). Participants underwent preoperative high resolution computed tomography (HRCT) of the mastoid. Postoperative cone beam computed tomography (CBCT) imaging was performed within five hours after surgery. A radiological tool as developed by Advanced Bionics was used to analyze implant array position by pre- and postoperative imaging in terms of occurrence of STL and insertion depth (aDOI). STL rates and aDOI were compared between all groups.

Results: Analyses pre- and postoperative imaging of 43 participants have been performed thus far, of which 34 (9/11 in group 1; 7/10 in group 2; 8/10 in group 3; 10/12 in group 4) were

successful. Preliminary results showed that STL was observed in 9/34 (26.5%) successfully analyzed images. For group 1 (RW + LW) STL occurred in 1/9 participants (11.1%). For group 2 (CO + LW) STL occurred in 2/7 participants (28.6%). For group 3 (RW + PM) STL occurred in 3/8 participants (37.5%). For group 4 (CO + PM) STL occurred in 3/10 participants (30%). A mean cochlear width B of 6.93 (± 0.35) mm was found in the first 43 participants. A mean aDOI of 405 (± 45) degrees was found in the 34 successfully analyzed images. After completion of all 48 inclusions, between group analysis will be conducted of pre- and postoperative images to compare results between the four study groups.

Conclusions: To improve hearing preservation, preoperative planning before cochlear implantation surgery could potentially include the selection of an electrode model in combination with a surgical approach.

Post-Surgical Hearing Loss With Cochlear Implantation: Measures and Models

11:45 a.m. - 12:00 p.m.

Rachel Scheperle¹, Stephen Neely², Peizhe Wu¹, Marlan Hansen¹

¹*University of Iowa Hospitals and Clinics*, ²*Boys Town National Research Hospital*

Background: Preservation of acoustic hearing post cochlear implantation (CI) is achievable, though with variable success rates (Snels et al., 2018; Gantz et al., 2022). Post-surgical changes in hearing status can be categorized according to their underlying mechanism (e.g. middle ear sound transmission, cochlear transduction, or neural excitation). Identifying the responsible mechanism is critical to hearing preservation and influences audiological management. This project aims to evaluate the changes observed in longitudinal electrocochleography (ECochG) measurements in relation to behavioral thresholds, and to assess whether a cochlear model can provide insights regarding the identification of underlying mechanisms.

Methods: Forty-eight CI recipients with varying degrees of residual acoustic hearing are enrolled in a longitudinal study that includes serial measurements of behavioral thresholds and ECochG. ECochG were elicited at a supra-threshold level using alternating-phase tonebursts. The difference waveform was Fourier transformed. Both magnitude and phase at the stimulus frequency were tracked longitudinally to compare with behavioral thresholds. Exemplar data sets were chosen to compare with the cochlear model, with attention to changes in measurements elicited at 500-Hz.

A time-domain model of cochlear mechanics that includes a middle-ear preprocessing stage followed by a cochlear processing stage was used to produce a simulated ECochG (Neely and Rasetshwane, 2017). The model was extended to estimate the voltage at an intracochlear location, which was adjusted to fit individual ears, by including volume-conduction impedance to combine individual OHC current across the cochlea (van Gendt, 2020). Selected model parameters were then manipulated to produce a combination of middle ear conductive hearing loss (CHL) and outer hair cell (OHC) dysfunction that would simulate the 500-Hz threshold shift observed in the exemplar ears. The effects of these model manipulations on delay of the simulated ECochG were compared to the measured ECochG.

Results: Behavioral hearing thresholds are typically poorest in the first weeks after implantation and recover slightly across subsequent weeks or months. While ECoChG amplitude reductions follow roughly similar patterns as threshold shifts, delay changes could be either positive or negative. Threshold shifts and ECoChG delay changes were simulated by a specific combination of CHL and OHC dysfunction. The phase of middle-ear input impedance in the model was affected by CHL but not OHC dysfunction.

Conclusions: Changes in acoustic hearing status post CI reflect various underlying mechanisms. Although (partial) recovery of audiometric thresholds at early time points has been interpreted to suggest a transient conductive loss, comparison of ECoChG delay with model results suggest intracochlear contributions may also be present. In future work, middle-ear input impedance estimated from acoustic measurements could have value in distinguishing whether threshold losses are conductive or sensory.

Otogenetic Stimulation in a High-Resolution Model of Human Cochlea

12:00 p.m. - 12:15 p.m.

Siwei Bai¹, Albert Croner¹, Guanyu Chen¹, Lukas Driendl¹, Werner Hemmert*¹

¹*Technical University of Munich*

Background: Conventional cochlear implants (CIs) are one of the most successful achievements of medical technology. However, their performance is limited by the wide spread of electric current in the highly conductive perilymph inside scala tympani. As a solution, optogenetic cochlear implants (oCIs) may achieve a much more focal stimulation. As the cochlea in humans is embedded within the skull and thus not directly accessible to electrophysiological recordings, computational models of the human cochlea are indispensable to gain more insight into how oCIs excite fibers within the population of auditory nerve fibres and how the excitation patterns compare with conventional CIs.

Methods: An anatomically realistic model of the cochlea and auditory nerve was reconstructed from a high-resolution μ CT scan of a human cochlea with 6 μ m voxel size (Bai et al., 2019). In this preliminary study, we acquired only half of the basal turn of the cochlea with the nerve. We imported the model into Ansys Speos to perform Monte-Carlo ray-tracing simulations of light spread in the cochlea. Volumetric optical properties of the model tissues were based on Khurana et al. (2022), and surface properties were based on Ugnell and Öberg (1997) and Stelzle et al. (2010, 2011). Both waveguide-based and LED-based oCIs were simulated with an emission power of 30mW and a wavelength of 655nm, facing directly towards Rosenthal's canal. The incident irradiance values were obtained along the centerline of Rosenthal's canal, along the centerline between Rosenthal's canal and the edge of the spiral lamina, and along the auditory neural fibre closest to the emitter.

Results: The number of rays from the emitter has an impact on the convergence of the simulation outcomes, and we found that five million rays provided the optimal convergence for our simulations. Using more realistic surface properties, compared to the Fresnel Laws, waveguide-based oCIs produced a slightly more diffuse emission profile, whereas LED-based oCIs produced a slightly more focal profile. Their maximum irradiances were, however, all reduced.

Conclusions: The passive waveguide oCIs resulted in a much more focal and more intense stimulation at the region where the emitter was facing; the smaller the numerical aperture was, the stronger the effect it showed. While this type of emitter has a potential benefit of supreme spectral selectivity, it may be extremely sensitive to the implantation technique, as it requires precise orientation. In comparison, the LED-based oCIs resulted in a broader stimulation, and the strongest irradiance appeared not at the soma, where the emitter was facing, but at the peripheral axon.

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Uncovering Patterns in Post-Operative Cochlear Implant Hearing Outcomes Through Group Based Trajectory Modeling

12:15 p.m. - 12:30 p.m.

Connie Chang-Chien¹, Max Jiam¹, Yew Song Cheng¹, Nicole Jiam¹

¹*University of California - San Francisco*

Background: While machine learning methods have increasingly been applied to predict cochlear implant (CI) outcomes, they primarily focus on cross-sectional predictions rather than capturing the dynamic, longitudinal rehabilitation progress. This study aims to apply GBTM (group-based trajectory modeling) to longitudinal WRS data to uncover discrete rehabilitation tracks post-implantation, addressing a critical gap in personalized outcome monitoring and intervention.

Methods: A retrospective cohort study was conducted on 122 adult CI users with bilateral sensorineural hearing loss implanted at a tertiary academic center. The primary tracked outcome was word recognition score (WRS) per appointment from pre-operative baseline to 60 months post-implantation. Patient demographic information and bilateral pre-operative PTA and WRS for both implanted and contralateral ears were included as GBTM context. Missing data were imputed using forward and backward fill methods, and trajectories were standardized by converting each time point measurement to z-scores. Longitudinal trajectory analysis was performed using Gaussian Mixture Models (GMM) to identify distinct outcome groups by minimizing entropy via Bayesian Information Criterion (BIC), first with all patient data, then for two age-stratified groups (age at implantation < 60 and ≥60). Statistical analysis of covariate group differences was evaluated based on data distributions.

Results: GBTM identified three general trajectory groups across all patients (BIC=-8524.5, $p < 0.001$), with significant separation for age at implantation (Kruskal-Wallis statistic (KW)=7.10, $p=0.029$). For the < 60 age cohort (n=31), we identified three trajectory groups (BIC=504.9, $p < 0.001$) with significant non-zero slopes, where implanted ear pre-operative WRS was the only

significant predictor of group membership (KW=6.58, $p=0.037$). The implanted ear pre-operative WRS for < 60 sub-groups were 6.56 ± 11.30 , 17.60 ± 17.5 , and 26.42 ± 21.72 , with trajectory slopes of 0.231, 0.428, and 0.149 via least squares linear regression ($p < 0.05$). In the ≥ 60 age cohort ($n=91$), we identified four trajectory groups (BIC=780.6, $p < 0.05$), but only one with a significant non-zero slope (-0.286 , $p=0.003$). Notable features for the down-trending subgroup included a lower pre-implantation WRS in the implanted ear (17.79 vs 19.56-24.24), but having a similar contralateral ear PTA and WRS, implantation age, and demographic features as others in the same age cohort.

Conclusions: Younger adults (< 60) exhibited more predictable rehabilitation trajectories characterized by pre-implantation baseline hearing status, with those starting with lower pre-operative implanted ear WRS and private insurance demonstrating the most rapid early improvements. In contrast, older patients (≥ 60) exhibited greater rehabilitation heterogeneity, including a subgroup with poor post-CI adaptation and no distinguishing study demographic and clinical predictors besides a lower implanted ear pre-operative WRS compared to other patients in the same age cohort. These findings highlight that younger adult CI users may follow more uniform recovery pathways, while older adults may require closer longitudinal monitoring and individualized interventions. Incorporating trajectory-based models into clinical care could enhance risk stratification, guide rehabilitation strategies, and inform counseling about expected recovery patterns.

Podium 6: OTOF Gene Therapy Trial Updates and New Targets: GJB2 and STRC

10:30 a.m. - 12:30 p.m.

Meeting Room 209ABC, Floor 2

Moderators: Jeffrey R. Holt and Aara Patel

International Expert Consensus on Gene Therapy for Hereditary Hearing Loss: Based on Clinical Trials

10:30 a.m. - 10:45 a.m.

Xiaoyun Chen^{*1}, Xintai Fan¹, Lukas Landegger², Tobias Moser³, Fan-Gang Zeng⁴, Robert Nash⁵, Wade Chien⁶, Dan Jiang⁷, John Greinwald⁸, Manohar Bance⁹, Manuel Manrique Rodríguez¹⁰, Sang-Yeon Lee¹¹, Chen-Chi Wu¹², Barbara Vona¹³, Nicola Strenzke¹³, Dirk Beutner¹³, Nikul Amin⁵, James Arwyn-Jones¹⁴, Deepak Chandrasekharan⁵, Yen-Fu Cheng¹⁵, Xue Zhong Liu¹⁶, Shiming Yang¹⁷, Hao Wu¹⁸, Zhengmin Wang¹⁹, Huawei Li¹⁹, Jay Rubinstein²⁰, Lawrence Lustig²¹, Renjie Chai²², Zheng-Yi Chen²³, Yilai Shu¹

¹ENT Institute and Department of Otorhinolaryngology, Eye and ENT Hospital, Fudan University, ²Stanford University School of Medicine, ³Institute for Auditory Neurosciences and InnerEarLab, University Medical Center Göttingen, ⁴University of California Irvine, ⁵Great Ormond Street Hospital, ⁶Johns Hopkins School of Medicine, ⁷St. Thomas Hospital, Guy's and

St. Thomas' NHS Foundation Trust, ⁸Cincinnati Children's Hospital, University of Cincinnati College of Medicine, ⁹University of Cambridge, ¹⁰Clínica Universidad de Navarra, ¹¹Seoul National University Hospital and Seoul National University College of Medicine, ¹²Taiwan University Hospital, ¹³University Medical Center Göttingen, ¹⁴Great Ormond Street Institute of Child Health, ¹⁵Taipei Veterans General Hospital, ¹⁶University of Miami Miller School of Medicine, ¹⁷Chinese PLA General Hospital, Chinese PLA Medical School, ¹⁸Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, ¹⁹ENT Institute, Eye and ENT Hospital, Fudan University, ²⁰University of Washington, ²¹Columbia University Medical Center, ²²Southeast University, ²³Massachusetts Eye and Ear/Harvard Medical School

Background: Hereditary hearing loss is one of the most common disabling disorders in children and lacks effective pharmacological treatments. Recent breakthroughs in OTOF gene therapy clinical trials necessitate standardized frameworks to guide emerging therapies. This study aimed to establish the first international consensus on clinical application of gene therapy for hereditary hearing loss.

Methods: A modified Delphi process was conducted from March 2024 to March 2025, involving 46 multidisciplinary experts from several countries across otology, genetics, audiology, gene therapy, and hearing rehabilitation. After a systematic literature review, as well as integration of research and clinical expertise and experience, three iterative voting rounds (two anonymous surveys and one online consensus meeting) were performed. Statements required $\geq 75\%$ agreement for inclusion.

Results: From 9093 publications, 69 were used to draft and support the consensus statements. A total of 30 statements relevant to six domains achieved consensus on gene therapy for hereditary hearing loss, including ethical review (1 statement), patient selection criteria (12 statements), diagnosis and preoperative evaluation (9 statements), gene therapy drug delivery (4 statements), follow-up (3 statements), and post-treatment auditory and speech rehabilitation (1 statement).

Conclusions: This consensus provides the first globally endorsed framework for gene therapy in hereditary hearing loss. It standardizes clinical trial design and patient management, accelerating translation from research to practice while ensuring safety. The guidelines are immediately applicable to OTOF-related hearing loss and adaptable to other genetic forms.

Multicenter Gene Therapy for Otof-Related Deafness Followed up to 2 Years

10:45 a.m. - 11:00 a.m.

Yilai Shu^{*1}, Luoying Jiang¹, Xiaoting Cheng¹, Jun Lv¹, Yuxin Chen¹, Xiaoyun Chen¹, Liqin Zhang², Lei Han¹, Xin Zhang¹, Qi Cao¹, Daqi Wang¹, Wei Yuan³, Qin Wang⁴, Guodong Feng², Huawei Li¹, Zheng-Yi Chen⁵

¹ENT Institute, Eye and ENT Hospital, NHC Key Laboratory of Hearing Medicine, Institutes of Biomedical Sciences, State Key Laboratory of Medical Neurobiology and MOE Frontiers Center for Brain Science, Shanghai Key Laboratory of Gene Editing and Cell Therapy for Rare Diseases, Fudan University, ²Peking Union Medical College Hospital³Chongqing General

Hospital (The Affiliated Hospital of Chongqing University) ⁴The Second Xiangya Hospital, ⁵Harvard Medical School

Background: Autosomal recessive deafness 9 (DFNB9), caused by OTOF gene mutations, is characterized by prelingual or congenital, severe-to-complete bilateral hearing loss. While the benefit of gene therapy has been proven in a limited number of DFNB9 patients, its efficacy and safety across a broader age range and longer follow-up remain to be evaluated.

Methods: In this single-arm, multicenter trial conducted at 4 centers, patients aged ≥ 6 months with severe-to-complete hearing loss resulting from OTOF mutations were eligible. Patients receive a single cochlear administration of AAV1-hOTOF via the round window membrane at three vector dose groups. The primary endpoint was dose-limiting toxicity within 6 weeks. The secondary endpoint assessed efficacy, including auditory function and speech perception, and safety including adverse events.

Results: 31 DFNB9 participants (aged 1.0 to 32.3 years) received gene therapy, with a follow-up up to 2 years. No dose-limiting toxicities or serious adverse events were observed. Grade 3 events included decreased neutrophil count (3 of 31). Hearing recovered in 90% (28 of 31) of participants, with the improvement of the auditory brainstem response thresholds from > 96 dB at baseline to 55, 51, and 37 dB at 1, 1.5, and 2 years, respectively. Participants with hearing recovery exhibited an improvement in speech perception.

Conclusions: AAV1-hOTOF is well-tolerated and efficacious across a broader patient population, with sustained therapeutic benefits up to 2 years.

Safety and Efficacy of DB-OTO Gene Therapy in Children With Profound Deafness Due to Otoferlin Variants: Data From the Chord First-In-Human Registrational Trial

11:00 a.m. - 11:15 a.m.

Eliot Shearer^{*1}, Manohar Bance², Daniela S. Carvalho³, John H. Greinwald Jr.⁴, Steven A. Harvey⁵, Akira Ishiyama⁶, Evie C. Landry⁷, Hubert Löwenheim⁸, Lawrence R. Lustig⁹, Manuel Manrique¹⁰, Robert Nash¹¹, Rubén Polo¹², Cedric V. Pritchett¹³, Jay T. Rubinstein¹⁴, Ignacio del Castillo¹⁵, Jeffery J. Anderson¹⁶, C. Eduardo Corrales¹⁷, Tera M. Quigley¹⁶, Jonathon P. Whitton¹⁶, Vassili Valayannopoulos¹⁶

¹Harvard Medical School, ²University of Cambridge, ³University of California, ⁴Cincinnati Children's Hospital, University of Cincinnati, ⁵Medical College of Wisconsin, ⁶David Geffen School of Medicine UCLA, ⁷Nemours Children's Health, Jacksonville, ⁸University of Tübingen Medical Center, ⁹Columbia University Irving Medical Center, New York Presbyterian Hospital, ¹⁰University Clinic of Navarre, ¹¹GOSH Institute of Child Health, University College London, ¹²Hospital Ramón y Cajal, ¹³Nemours Children's Health, Orlando, ¹⁴Virginia Merrill Bloedel Hearing Research Center, Seattle Children's Hospital, University of Washington, ¹⁵Servicio de Genética, Hospital Universitario Ramón y Cajal, Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), ¹⁶Regeneron Pharmaceuticals, Inc., ¹⁷Brigham and Women's Hospital, Harvard Medical School

Background: Genetic deficiency of otoferlin (OTOF) disrupts synaptic transmission by the auditory sensory hair cells, causing congenital deafness. Children with OTOF-related deafness are typically treated with cochlear implants. DB-OTO is a gene therapy based on a dual adeno-associated virus 1 which delivers human OTOF complementary DNA regulated by a hair cell-specific promoter.

Methods: The CHORD trial is an open-label, single-arm, first-in-human registrational study evaluating DB-OTO (NCT05788536). Twelve children with profound deafness carrying biallelic OTOF pathogenic variants received an intracochlear DB-OTO infusion (7.2×10^{12} vector genomes/ear) unilaterally or bilaterally. The primary efficacy endpoint was the achievement of average thresholds ≤ 70 decibel hearing level (dBHL) by behavioral pure tone audiometry (PTA) at Week 24, a clinical standard that generally allows for natural acoustic hearing without the need for cochlear implants. The key secondary endpoint was the presence of auditory brainstem response to a 90 decibel normal hearing level (dBnHL) click stimulus. Incidence and severity of adverse events was a primary safety endpoint. Laboratory, limited vestibular, and immunogenicity testing were also performed.

Results: Following single DB-OTO administration, 11 of 12 participants (92%) with profound deafness at baseline were able to hear for the first time. Nine of 12 participants (75%) achieved clinically significant hearing improvements ($P < 0.0001$), enabling natural hearing, meeting the primary and key secondary endpoints, and obviating the need for cochlear implants. Six of 12 participants (50%) were able to detect soft speech without assistive devices (average PTA ≤ 45 dBHL), and 3 of 12 participants (25%) achieved complete normalization of hearing sensitivity (≤ 25 dBHL) by Week 24 as measured by average PTA. (Average PTA ≤ 25 dBHL). Preliminary data from 1.5 years of follow-up indicate sustained or improved hearing, as well as improvements in speech and language development. No DB-OTO-related adverse events were recorded.

Conclusions: In this first-in-human registrational gene therapy trial, DB-OTO resulted in significant hearing improvements in participants with OTOF-related deafness. DB-OTO administration enabled natural acoustic hearing for the first time, and normalized hearing sensitivity in some participants.

Marmoset Monkeys With a Non-Mosaic Disruption of the Otof Gene as a Model of Human Deafness

11:15 a.m. - 11:30 a.m.

Mara Uhl¹, Tobias Kahland², Dimitri Leonid Lindenwald², Marcus Jeschke³, Kathrin Kusch⁴, Olena Tkachenko Eikel², Nancy Rüger², Charis Drummer², Bettina Wolf⁴, Fritz Benseler⁵, Nils Brose⁵, Rüdiger Behr², Tobias Moser⁴

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*Center*⁴*Institute for Auditory Neuroscience, University Medical Center Goettingen*⁵*Max Planck Institute for Multidisciplinary Sciences*

Background: About 5% of the world's population suffers from disabling hearing loss. In newborns, 50% of hearing loss is attributed to genetic factors. One of the deafness genes is OTOF, causing the auditory synaptopathy DFNB9, which makes up 2-8% of congenital hearing loss. Within the last year's great progress has been made in gene therapy of DFNB9 from proof of concept in mice, to favorable biosafety data in non-human primates (NHP) and promising results in clinical trials. Yet questions of safety, efficacy, and longevity of different OTOF gene therapy strategies remain to be answered. Therefore, the need of a NHP model of DFNB9 is eminent. Moreover, such a model could be used to study other methods of hearing restoration, bridging the gap between mouse experiments and clinical trials.

Methods: Marmoset oocytes harvested upon ovary stimulation, were used for in vitro fertilization. Subsequently these fertilized oocytes were injected with Cas9 and four guide RNAs. At the 6 to 12-cell-stage the embryos were transferred to the uterus of surrogate mothers. After birth PCR genotyping of all germ layers was performed and the PCR products were subcloned to obtain single-clone DNA sequences. The phenotype of the animals was characterized, monitoring the weight development of the animals, analyzing their vocalizations and testing their hearing. Three to four vocalization recordings each lasting 10 min were performed at two to four weeks after birth. At 6 months of age, auditory brainstem responses to clicks and pure tones of different frequencies were probed and DPOAEs were acquired. Furthermore, immunohistofluorescence staining was performed on the organ of Corti of one of the OTOF-knockout marmosets.

Results: Two marmosets were born carrying biallelic mutations of OTOF, resulting in a knockout. They were born naturally and cared for normally by their parents. The OTOF knockout had no effect on the overall development. The mutations were detected in all germ layers, indicating the absence of mosaicism. Auditory brainstem recordings revealed a profound hearing loss, while the detection of otoacoustic emissions indicated the functionality of the cochlea upstream of synaptic sound encoding. The structural integrity of the organ of Corti was confirmed by immunohistofluorescence. Moreover, this method confirmed the loss of OTOF within the inner hair cells. Preliminary data suggest that the vocalizations of the deaf marmosets were largely unaltered compared to age-matched WT animals. They used the full vocal repertoire, and their vocal activity was similar to WT marmosets.

Conclusions: We generated OTOF-knockout marmosets via CRISP/Cas9 injection into in vitro matured and fertilized oocytes. OTOF-knockout marmosets present a non-mosaic NHP model for DFNB9. They provide a model for testing and optimizing safety, efficacy, and longevity of OTOF gene therapy and serve the development of other methods for hearing restoration such as optical cochlear implants.

SENS-601, a Novel Adeno Associated Vector-Based Gene Therapy as a Treatment for the Autosomal Recessive Non-Syndromic Deafness 1A (DFNB1A)

11:30 a.m. - 11:45 a.m.

Arnaud Giese^{*1}, Guillaume OLIVIER¹, Christophe Tranv Van Ba¹, Sandra Pierredon¹, Anne-Gabrielle Harrus¹, Pierre Rambeau¹, Lise Barrot¹, Anais Pages¹, Laurene Heriaud¹, Pauline Liaudet¹, Audrey Broussy¹, Selma Dadak¹, Christine Le Bec¹, Emilie Bousquet¹, Anne-Valérie

HERITIER², Charlene Vaux¹, Amrit Kaur ESTIVALET-SINGH², Charlene JOSEPHINE¹, Andrea Lelli², Muriel Sudres², Nicolas Michalski², Geraldine Petit¹, Rafik Boudra¹, Christine Petit², Laurent Desire¹

¹SENSORION SA, ²Institut de l'Audition/ Institut Pasteur

Background: The estimated prevalence of severe or profound deafness in humans is 1/1000 neonates, and genetic factors account for half of the cases. Pathogenic variants of GJB2, the gene encoding Connexin 26 (Cx26), are involved in 50% of congenital deafness and are mostly associated with autosomal recessive non-syndromic DFNB1A. In the cochlea GJB2, is largely expressed in the majority of supporting cells (SCs) of the neuroepithelium but not in sensory hair cells (SHCs).

Today, gene therapy strategies for autosomal recessive forms of deafness have shown, in clinical trials, promising hearing recovery. In some cases, some patients tend to show more rapid improvements in auditory and speech performance than those with cochlear implants. To this aim, we have developed SENS-601, an AAV vector for DNFB1A with proprietary cis-regulatory elements for a safe and targeted transgene expression. SENS-601 efficiently transduces human cell lines to produce Cx26 protein that is adequately addressed to the plasma membrane and allows functional gap junctions.

Methods: Several conditional mouse models were generated to elude embryonic mouse lethality caused by complete loss of Gjb2 expression, mimicking the most common forms of DFNB1A and severe/profound hearing loss and a deficit of the endocochlear potential. Intracochlear injections of SENS-601 lead to improvement of hearing thresholds as early as 3 weeks post-injection in a dose-dependent manner.

Results: Microscopic examination confirmed that SENS-601 efficiently and adequately restores Cx26 expression. Dose-response experiments show that intracochlear SENS-601 injection is safe over a wide range of doses administered and provides a significant therapeutic benefit in DFNB1A mice. Restoration of the endocochlear potential is currently being addressed.

Early tolerability and biodistribution of SENS-601, or SENS-601-FLAG, studies were conducted in mice and Non-Human Primates (NHP) after injections through the round window (RW). Transgene expression was observed in most cells that naturally express Gjb2 along the tonotopic axis of the cochlea, with good local and systemic tolerability. In particular, no SHCs express the transgene, confirming the specificity of the expression cassette in both species. Furthermore, SENS-601 long-term safety studies conducted in wild-type mice demonstrate persistence of transgene expression with no impact on auditory function or cochlear cytoarchitecture. High-dose intravenous injection of SENS-601 does not induce any adverse events or changes in behavior. A combined 3- and 6-month GLP toxicology and biodistribution study of SENS-601 is currently ongoing in NHP, following a single intra-cochlear injection administered using the surgical procedure and injection device intended for clinical use, to evaluate the local tolerability and safety of SENS-601.

Conclusions: Overall, efficacy, biodistribution and early safety data package support SENS-601 development to restore physiological hearing in DFNB1A patients

Capsid and Promoter Engineering for AAV Gene Therapy and Genome Editing in GJB2-Related Hearing Loss

11:45 a.m. - 12:00 p.m.

Kazusaku Kamiya^{*1}, Daisuke Arai¹, Hidekane Yoshimura², Shin-ya Nishio², Sho Kanzaki¹, Yutaka Takumi², Katsuhisa Ikeda¹, Shin-ichi Usami²

¹*Juntendo University Faculty of Medicine*, ²*Shinshu University School of Medicine*

Background: Mutation of GJB2 (encoding Gap Junction Beta 2) is the most frequent cause of hereditary deafness worldwide and accounts for up to 50% of non-syndromic sensorineural hearing loss. GJB2 encodes connexin 26, a component of cochlear gap junctions that help maintain ion balance in the cochlea. We previously demonstrated that GJB2 deficiency in mice impairs auditory function (Kamiya, *J. Clin. Invest.*, 2014) and that immediate postnatal delivery of wild-type GJB2 to the inner ear via adeno-associated virus (AAV) vector restored the hearing loss in mice (Iizuka, *Hum. Mol. Genet.*, 2015). However, delivery of GJB2 to the mature cochlea did not restore hearing. The early postnatal period in mice corresponds to the embryonic period of inner-ear developmental in humans. Thus, to develop a feasible clinical approach for human gene therapy, mature mice should be used for delivery of AAV to the inner ear. To achieve this, the development of an optimal AAV vector platform for GJB2-related hearing loss is indispensable.

Methods: We generated the several AAV capsids that effectively infect cochlear supporting cells by shuffling capsid sequences between wild serotypes or directed evolution with AAV library. And the promoters were modified to express only in GJB2 expressing cells and form proper gap junctions. Then the vectors were tested with adult GJB2 deficient mice (Kamiya, *J. Clin. Invest.*, 2014) and our supporting cell model derived from human patient iPS (induced pluripotent stem) cells with typical GJB2 mutation (Fukunaga, *Stem Cell Reports*, 2016, *Hum. Mol. Genet.* 2021). As part of a preclinical study, we administered the novel AAV vector to non-human primates (NHPs).

Results: We generated novel AAV vectors by shuffling capsid sequences between wild serotypes and selecting those that effectively infect cochlear GJB2-positive cells. To avoid transgene expression in non-target cells such as hair cells, we developed a specific promoter for GJB2-expressing cells. Novel AAV-mediated delivery of GJB2 significantly restored hearing loss in mature GJB2-deficient mice, and gap junction recovery was confirmed in both animal (GJB2-deficient mice) and cellular models (patient iPS cell-derived models). In NHPs administered with our novel AAV vector, AAV-derived protein expression in the target cells (cochlear supporting cells and fibrocytes) was broadly detected.

Conclusions: These findings indicate that our AAV vector carrying the novel capsid/promoter could serve as an optimal platform for gene therapy of GJB2-related hearing loss.

Dual AAV Gene Therapy Achieves Sustained Recovery of Hearing and Auditory Processing in a DFNB16 Mouse Model

12:00 p.m. - 12:15 p.m.

Sepideh Iranfar¹, Sophie Bagur², Chloé Felgerolle², Maxence Cornille², Hélène Le Ribeuz², Marie Giorgi², Sara Jamali², Amel Saoudi², Marie-José Lecomte², Vincent Michel², Brice Bathellier², Saaïd Safieddine^{*2}

¹Université Paris Cité, Institut Pasteur, AP-HP, INSERM, CNRS, Fondation Pour l'Audition, Institut de l'Audition, IHU reConnect, Paris, F-75012, France, Sorbonne Université, Ecole Doctorale Physiologie, Physiopathologie et Thérapeutique, Paris, France, ²Université Paris Cité, Institut Pasteur, AP-HP, INSERM, CNRS, Fondation Pour l'Audition, Institut de l'Audition, IHU reConnect, Paris, F-75012, France

Background: DFNB16, the second most common cause of hearing loss, accounting for ~11% of reported cases, with a carrier frequency of 1.6–1.8% in the general population. It is caused by mutations in the stereocilin (STRC) gene which encodes a protein predominantly expressed in outer hair cells (OHCs). STRC plays a crucial role in maintaining OHC bundle cohesion and in enabling their function as cochlear amplifiers. Loss of STRC function results in mild-to-moderate deafness in humans. Despite its prevalence, no curative treatment exists for DFNB16. *Strc*^{-/-} mice, which lack stereocilin, display severe to profound deafness and constitute a relevant preclinical model for DFNB16 (Verpy et al., 2008). Here, we developed an AAV-based gene therapy specifically targeting OHCs to achieve robust and sustained restoration of hearing function and central auditory processing.

Methods: Because the STRC cDNA exceeds the packaging capacity of a single AAV vector, we employed a dual-AAV delivery strategy, previously validated for DFNB9 (Akil et al., 2019). STRC expression and structural integrity of OHCs were analyzed by immunohistochemistry and confocal imaging and scanning electron microscopy. Hearing function was evaluated by recording distortion product otoacoustic emissions (DPOAEs) and auditory brainstem responses (ABRs) responses, which assess OHC function and synchronized neural activity from the auditory nerve to the brainstem, respectively. Auditory perception was evaluated through a Go/No-Go auditory frequency discrimination task combined with psychometric analysis.

Results: Dual-AAV-mediated *Strc* gene delivery successfully restored stereocilin expression, OHC bundle architecture, and stereocilia attachment to the tectorial membrane in *Strc*^{-/-} mice. This structural recovery led to the restoration of cochlear amplification and hearing to near-normal thresholds, as confirmed by DPOAE and ABR measurements. Importantly, dual-AAV administration up to postnatal day 5 (P5) restored auditory thresholds to levels not significantly different from those of wild-type controls, indicating a critical developmental window for effective intervention. Finally, behavioral assessments further demonstrated that treated *Strc*^{-/-} mice regained auditory frequency discrimination abilities comparable to wild-type littermates. These perceptual improvements were maintained for at least 100 days post-treatment, indicating long-term functional rescue at both peripheral and central auditory levels.

Conclusions: This study provides the first proof-of-principle that AAV-based gene therapy can restore OHC function, cochlear amplification, and central auditory perception in a DFNB16 model. Our findings highlight the therapeutic potential of dual-AAV approaches for STRC-related hearing loss and underscore their translational relevance for clinical application in humans.

In Vivo Evolved AAV Variants Enable Dual-AAV Stereocilin Delivery and Outer Hair Cell Rescue

12:15 p.m. - 12:30 p.m.

Josephine Wilkes¹, Jennifer Marx¹, Nico Jaeschke¹, Lisa Prager¹, Sabrina Just¹, Philipp John-Neek¹, C Wiehlmann¹, A Gardenstaeeter², Matthias Gerlitz², Erdem Yildiz², Athanasia Warnecke¹, Odett Kaiser¹, Hinrich Staecker^{*3}, Hildegard Buening¹

¹Hannover Medical School, ²Vienna General Hospital, Medical University of Vienna,

³Univeristy of Kansas Medical Center

Background: Hearing loss (HL) affects ~20% of the global population, yet current interventions are limited to hearing aids and cochlear implants. Gene therapy offers a promising alternative by directly addressing the molecular causes of HL. Adeno-associated virus (AAV) vectors are powerful gene transfer tools, but most variants used in the inner ear originate from libraries selected in other tissues or in silico designs. Directed evolution in the inner ear can generate optimized capsids with higher tropism for auditory cell types. This is particularly relevant for DFNB16, the second most common cause of autosomal recessive non-syndromic HL, resulting from mutations in the stereocilin (STRC) gene. The large size of STRC necessitates dual-AAV strategies, such as RNA trans-splicing systems, to enable full-length gene delivery.

Methods: AAV peptide display libraries based on AAV1, AAV2, and AAV6 with random 7-mer insertions in capsid region VIII were screened in vivo in adult mice (n=4/library) using alternative administration routes, requiring vectors to overcome robust biological barriers. High-throughput sequencing identified enriched capsids, which were produced as vectors carrying a dTomato reporter and tested in mice and pigs. For therapeutic application, the most promising OHC-targeting capsid (Var1) was employed in a dual-AAV system encoding split STRC genomes (dual-AAV1.Var1). Strc-deficient mice were treated at one month of age, and auditory brainstem responses (ABRs), stereocilia morphology, and transgene expression were assessed up to six months post-treatment.

Results: Selection enriched distinct capsids, with AAV2-derived variants accumulating up to 5% and AAV1-derived up to 2.5%. Top variants showed improved transduction compared to parental serotypes, including efficient targeting of outer and inner hair cells, stria vascularis, and spiral ganglion neurons (SGNs). In pigs, two lead candidates (Var1, Var3) reproducibly transduced cochlear cells. Using Var1 in a dual-AAV strategy, full-length STRC expression was confirmed in vitro within 48 h. In vivo, treated Strc-deficient mice showed modest ABR threshold improvements: small recovery at 16 kHz after 7 weeks and significant shifts across all tested frequencies at 6 months, though thresholds remained above 60 dB, indicating moderate-to-severe HL. In contrast, untreated ears showed severe-to-profound HL (> 80 dB). Whole-mount imaging revealed partial stereocilia recovery on 50–80% of OHCs in treated ears, and FLAG immunostaining confirmed STRC expression in OHCs, with additional expression in SGNs and spiral ligament, likely reflecting mRNA regulatory sequences.

Conclusions: This work establishes a panel of inner ear-optimized AAV variants with distinct tropisms and demonstrates the first in vivo application of dual-AAV STRC system in mature Strc-deficient mice. While hearing recovery was partial, the combination of OHC transduction, stereocilia rescue, and stable STRC expression validates the therapeutic potential of dual-AAV

delivery for DFNB16. These findings highlight the feasibility of tailoring AAV capsids for the inner ear and advance gene therapy approaches for large-gene hearing loss disorders.

Young Investigator Symposium 1: Real-World Acoustic Environments and Their Impact on Hearing and Well-Being

10:30 a.m. - 12:30 p.m.

Meeting Room 208ABC, Floor 2

Real-World Acoustic Environments and Their Impact on Hearing and Well-Being

Erika Skoe, *University of Connecticut*

Emily Spitzer, *New York University Grossman School of Medicine*

The Daily Auditory Environments of People With Tinnitus

Erika Skoe, *University of Connecticut*

Individual Abstract: Background:

Tinnitus is the sensation of sound in the absence of an external stimulus. The connection between long-term noise exposure and tinnitus has been the target of many studies, but little attention has been given to noise exposure patterns after the onset of tinnitus. A deeper understanding of the auditory environments of people with tinnitus is needed to aid the clinical management of tinnitus.

Methods:

For a week, participants (n=108) wore a personal noise dosimeter during all waking activities to measure their daily noise exposure. Participants also kept a journal of their activities during the week. The total sample size includes both tinnitus (n=61) and control groups (n=56). Dosimeter data were analyzed first using summative metrics of daily sound exposure, followed by functional data analysis (FDA) to reveal finer temporal differences in sound exposure between groups. The journal data were coded into 17 different categories to identify activities associated with noisy periods in the dosimeter record (> 85 dBA) and to compare groups with respect to their activity levels.

Results:

We found that daily personal noise exposure, measured using the time-weighted average sound pressure levels (LAeq,8h), was lower for the Tinnitus group relative to the Control group. Based on the dosimeter data, neither group was at ongoing risk of noise-induced hearing loss, as

average daily sound levels did not exceed 75 dB LAeq,8h for either group. Yet, the Tinnitus group spent less time engaged in noisy activities (> 85 dBA) compared to the Control group, with notable differences for home and workplace settings. However, overall, the groups did not differ in the total overall time they spent in these settings during the week of dosimetry. The FDA revealed that group differences were maximal around noon and evenings, periods when an individual is likely to have more control over their environment.

Conclusions:

Overall, we find that people with tinnitus tend to have quieter environments, suggesting that they lead quieter lives. Differences between groups may be explained by greater awareness of the risks of noise exposure in the tinnitus group or decreased sound tolerance among this group. Ongoing work seeks to replicate findings using smart Watch technology. One of the primary advantages of smart Watch technology is that, unlike personal noise dosimeters, it can provide data on headphone usage. Preliminary data will be presented.

Cultural Differences in Listening Environments Between Hispanic and White Non-Hispanic Cochlear Implant Users

Emily Spitzer, *New York University Grossman School of Medicine*

Individual Abstract: Introduction: Cochlear implant (CI) speech processors can track usage via “data logging”, including the number of hours on air, time spent in different acoustic environments and loudness levels. These data are important, as high noise levels and poor signal to noise ratios (SNRs) can decrease speech intelligibility, impairing communication and speech perception outcomes. Louder environments can have a detrimental impact on learning and cognition and have been associated with both socioeconomic disparity and cultural factors. A study found that normal hearing Latinx-identifying college students experienced higher levels of environmental noise and lower SNRs, possibly due to cultural importance of collectivism and music listening at louder levels. This study investigates whether similar listening patterns exist in CI users and examines how data logging variables relate to speech perception outcomes.

Methods: Retrospective chart review of CI patients at a tertiary medical center in New York City identified 30 Hispanic and 31 White non-Hispanic adults for further review. Demographic variables were compiled and home zip codes were used to obtain population-based socioeconomic data. Outcome measures included speech perception scores (in noise and quiet) conducted in the participant’s primary language and data logging information extracted from the CI speech processor. The datalog included hours of total use and time spent in different listening environments, classified by the CI software into sound levels (in dBA) and sound scenes (noise, quiet, speech, music, and “other”).

Results: There were no significant differences detected in total hours of CI usage, age at CI, or gender between the Hispanic and White non-Hispanic groups. There was a significant difference in the percentage of time spent in each scene: Hispanic Americans spent more time in “speech in noise”, “music”, and “noise”; non-Hispanics spent more time in “quiet”. Hispanic participants lived in neighborhoods with higher population densities, which correlated to higher CI sound

levels (dBA > 70) in the environment. The percentage of time spent in noise positively correlated with sentence-in-noise perception scores for Hispanic participants, but not for White non-Hispanic participants.

Conclusion: Cultural differences appear to exist between Hispanic and White non-Hispanic listening environments, like normal hearing listeners. While device use was similar, the Hispanic group had greater noise exposure, which was linked to better speech-in-noise performance. This relation may suggest an auditory training mechanism, reinforced by cultural values. Audiologists should consider cultural background and listening preferences when counseling CI users, as these may influence performance and adaptation to noisy environments.

Linking Hearing Ability, Acoustic Environments, Hearing Aids, and Social-Emotional Health Outcomes

Erik Jorgensen, *University of Wisconsin-Madison*

Individual Abstract: Background

Hearing loss is linked to increased loneliness and depression, but the mechanisms behind this association remain unclear. A leading hypothesis is that hearing loss leads to social-emotional difficulties by changing hearing-related behaviors—particularly by prompting people to avoid speech-in-noise (SiN) environments, which are often social and especially challenging for individuals with poor speech perception in noise (SPIN). This study tested that hypothesis by examining the relationships among hearing, hearing aid use, hearing-related behaviors, and social-emotional health.

Methods

The study included 54 younger adults with typical hearing, 55 older adults with hearing loss who did not use hearing aids, and 52 older adults with hearing loss who did. Participants completed standardized measures of SPIN, depression, and loneliness. They also wore body-worn recorders near the head for all waking hours for one week (younger adults) or two weeks (older adults). A deep neural network classified the acoustic environment in 0.5 s intervals, and the percent of time in each environment was computed. Descriptive statistics and structural equation modeling were used to analyze moderated mediation models linking acoustic environments, SPIN, loneliness, and depression.

Results

Younger adults spent the most time in SiN environments. Surprisingly, older hearing aid users spent significantly less time in SiN and more time in silence compared to non-users. Among younger adults, more time in SiN was associated with lower loneliness, especially for those with better SPIN, which indirectly reduced depression. For older non-users, a similar pattern emerged: more SiN exposure was associated with lower loneliness, especially with better SPIN. In contrast, for hearing aid users, more time in SiN was linked to greater loneliness. SiN exposure and SPIN ability were not associated with depression in older adults. An “environment-person fit” effect was also observed: those with poor SPIN who spent less time in SiN had lower

loneliness. Among older adults, time in SiN, SPIN ability, hearing aid use, and loneliness were all related to perceived hearing-related disability.

Conclusions

Acoustic environments vary by age and hearing status, with implications for social-emotional health. In younger adults, SPIN moderates the link between SiN exposure and loneliness, which in turn affects depression. Among older adults, hearing aid use does not mitigate loneliness or increase time in SiN; hearing aid users had poorer SPIN, spent less time in SiN, and reported higher loneliness than non-users. Poor SPIN and perceived hearing disability likely underlie avoidant hearing-related behaviors, pursuit of hearing aids, and loneliness.

Acoustic Environments for Children Who Are Deaf or Hard of Hearing

Carlos Benitez-Barrera, *University of South Florida*

Individual Abstract: Objectives:

Children who are Deaf or Hard of Hearing (CDHH) who develop spoken language are at increased risk for language delays. These delays are influenced by hearing-related factors (e.g., age of amplification) and reduced exposure to caregiver language input. Emerging models of language acquisition emphasize that environmental noise also limits access to caregiver input, further impacting language development. Our previous work showed that real-world speech-to-noise ratios (SNRs) predict language outcomes in children with typical hearing, beyond the effect of caregiver talk. Building on this, we propose that CDHH exposed to both limited input and poor acoustic access face the highest risk for language delays.

This presentation underscores the importance of assessing the quality of CDHH children's acoustic environments. Specifically, we highlight: (1) how wearable audio recording devices can objectively quantify children's auditory experiences, including access to caregiver input, and (2) how these experiences relate to language development.

Design:

We are recruiting 40 preschool CDHH (ages 3–6) and an age-matched control group with typical hearing. Our goal is to develop and validate a novel Speech Accessibility Index (SAI), which quantifies children's access to spoken language in everyday settings. The SAI integrates two metrics: (a) each child's Speech Reception Threshold (SRT) in noise, and (b) SNRs calculated from daylong LENA recordings collected at home. The SAI estimates the percentage of conversations with SNRs above the child's SRT, serving as a proxy for real-world language access. This approach builds on our prior work and extends conventional measures of language exposure to include the quality of acoustic input available to the child.

Results:

To date, we have collected data from 20 children (10 CDHH). Preliminary analyses indicate that both caregiver input and access to language (as indexed by the SAI) are significantly associated with language outcomes when combining both groups. We expect that, with the full dataset, both predictors will remain independently associated with language skills, even after controlling for relevant covariates.

Conclusions:

The SAI captures dimensions of children's language environments that go beyond caregiver speech alone, offering clinical value in assessing real-world speech access. It shows promise as a screening tool for identifying CDHH at risk for language delays and for informing home-based interventions (e.g., remote microphone systems) aimed at enhancing children's language access and outcomes.

Work supported by NIH-NIDCD (R21 DC022038) to C.R. Benítez-Barrera

Poster Session II with Coffee

1:30 p.m. - 3:00 p.m.

Ballroom A, Floor 3

Due to the policies of the Puerto Rico Convention Center, the Poster Hall will be available to attendees between the hours of 7AM – 11PM (local time), with a 1- hour break every day between 11AM and 12 PM (Noon) to facilitate setup for the next poster session. This is an appreciated extension of their normal permitted hours. ePosters are available on the conference website and the ARO MWM mobile app. and nbsp;

SU1. Silence and Solitude: Effects of Noise Exposure and Social Isolation on Serotonergic Function and Behavior

Megan Walker¹, Jessica Bush¹, Lauren Hadland¹, Laura Hurley*¹

¹*Indiana University*

Background: The social isolation that can accompany hearing impairment in aging co-occurs with other health concerns, including depression, anxiety, and cognitive decline. A contributing factor to these negative outcomes may be dysfunctional communication driven by deficits in auditory processing and perception. Animal models of these related risk factors can help elucidate the neural mechanisms underlying these functional challenges. Serotonin-auditory interactions are responsive to both hearing loss and social isolation in mice.

Methods: We therefore assessed the effects of noise exposure and a period of individual housing on serotonergic function and social and open field behaviors in female CBA/CaJ mice, using a factorial design. At 6 weeks of age, mice were exposed to noise from 8-16 kHz at 100 dB, or to sham treatment, for 2 hrs. At 11 months, mice were placed in individual housing for 4 weeks or maintained in social groups, after which they were placed in an opposite-sex social interaction with socially housed male partners. Following brain fixation and slicing, sections were labeled with antibodies for tryptophan hydroxylase plus c-Fos, or the serotonin transporter (SERT).

Results: Social isolation had strong effects on behavior, elevating vocal behavior in both the female subjects and their male partners, and increasing time in the edges of the open field. In contrast to previous studies in younger mice, social isolation did not alter the density of SERT+ fibers in the inferior colliculus or neurons double labeled for c-Fos and TPH in the dorsal raphe nucleus (DRN). Although noise exposure significantly elevated ABR thresholds, it did not affect vocal behavior or SERT+ density. Noise exposure did increase the density of neurons double

labeled for c-Fos and TPH in the dorsal raphe nucleus. However, the effect of noise was significant only in mice in the isolated treatment group. Further, the density of double labeled neurons in the DRN correlated with ABR threshold and with USV number.

Conclusions: These findings suggest that noise exposure and social isolation interact to influence the activity of serotonergic neurons during social interaction.

SU2. How Reverberation Shapes the Subcortical Encoding of Speech in Aging and Hearing Loss

Ebtesam Sajjadi*¹, Samira Anderson¹

¹*University of Maryland*

Background: Everyday communication often occurs in environments with reverberation, such as classrooms, restaurants, and auditoriums. Mild reverberation levels can enhance overall speech audibility, but excessive levels of reverberation blur temporal and spectral cues and deteriorate speech understanding. This difficulty is more pronounced for older adults and individuals with hearing loss. The purpose of this study was to examine how reverberation affects subcortical speech encoding to gain a better understanding of factors that may contribute to perceptual declines.

Methods: The current project uses frequency-following responses (FFRs) to investigate the subcortical neural encoding of reverberation in older adults. Prior work has shown that age-related declines in temporal precision particularly compromise encoding of dynamic formant transitions in speech, whereas steady-state vowel segments may be relatively preserved. Therefore, FFRs have been recorded to the syllables /da/ and /ah/ to represent dynamic (formant transition) and static (steady-state vowel) components of speech, respectively. Stimuli are presented under no reverberation, short decay (RT60 = 0.46 s), and long decay (RT60 = 2s) conditions. FFRs have been recorded from older adults with normal hearing (ONH) and mild to moderate hearing loss (OHI) at 80 dB SPL. Responses are analyzed using phase locking to the envelope and temporal fine structure and stimulus-to-response correlations to assess the fidelity of neural encoding across different time regions of the speech signal.

Results: Preliminary results show that reverberation reduces phase locking and decreases stimulus-to-response correlations for both /da/ and /ah/ stimuli. Early findings suggest that long decay reverberation particularly weakens encoding of the transition region in /da/. This result is consistent with the previous findings that dynamic cues are more vulnerable to temporal smearing. While no statistically significant group effects have yet been observed, OHI participants generally show weaker envelope encoding under reverberant conditions than ONH participants.

Conclusions: These findings provide initial evidence that reverberation exacerbates age-related declines in neural precision, particularly for dynamic speech regions. Ongoing data collection will provide evidence about the differential effects of reverberation on steady-state and transition regions, and whether OHI listeners are at greater risk for degraded neural synchronization. This work aims to increase our understanding of how reverberation shapes subcortical encoding in aging populations and propose future strategies for improving the ability of this population to understand speech in acoustically complex environments.

SU3. Vascular Senescence and Microcirculatory Pathology as Contributors to Age-Related Hearing Loss

Marta Konopka^{*1}, Maria Luque¹, Rudolf Glueckert¹, Elisabeth Pechriggl¹, Erich Brenner¹

¹*Medical University of Innsbruck*

Background: Age-related hearing loss (ARHL) is one of the most prevalent sensory disorders in the elderly and is closely associated with cognitive decline and reduced quality of life. Increasing evidence suggests that vascular dysfunction, including endothelial alterations, chronic low-grade inflammation, and systemic vascular remodeling, contributes to cochlear degeneration. Human-based studies are required to clarify these mechanisms in the context of aging and diabetes.

Methods: Human postmortem specimens from donors older than 60 years were analyzed, including cochlea, internal carotid artery, kidney, and skin. Donors with type 2 diabetes mellitus were compared with non-diabetic controls. Tissues were processed using conventional histology and immunohistochemistry to assess four domains: vascular aging, low-grade inflammation, microangiopathies, and macroangiopathies. For this purpose, α -smooth muscle actin (α -SMA) was used to evaluate vascular smooth muscle remodeling, CD31 to assess endothelial integrity and vascularization, and CD68 to detect macrophage infiltration. Quantitative image analysis was applied where tissue quality allowed, enabling comparison of vascular remodeling, endothelial changes, and inflammatory burden across diabetic and non-diabetic groups.

Results: Macrovascular remodeling in the carotid artery was characterized by thickened media and pronounced intimal hyperplasia, consistent with advanced atherosclerotic change. Microvascular alterations were observed in cochlear and systemic tissues, including focal basement membrane irregularities and altered vascular density. A trend toward higher CD31 expression was detected in diabetic donors compared with non-diabetic controls, likely reflecting compensatory endothelial proliferation and remodeling in the setting of diabetic vasculopathy. Inflammatory infiltration was consistently observed, with CD68-positive macrophages located in arterial walls and cochlear regions. Quantification demonstrated higher macrophage infiltration in diabetic donors, supporting the association between chronic low-grade inflammation and vascular pathology. These findings currently represent trends, but with a larger donor database we expect not only confirmation of these patterns but also significant differences to emerge, clarifying the extent of vascular involvement in ARHL. Comparable alterations in kidney and skin underline the systemic nature of these changes.

Conclusions: By integrating inflammatory, microvascular, and macrovascular findings across cochlea and systemic tissues, this study supports the concept that ARHL represents a manifestation of systemic vascular aging rather than an isolated disorder of the inner ear. All findings will be correlated with progression of age-related hearing loss, providing a translational framework that links vascular pathology to auditory decline. These results highlight vascular dysfunction as a promising therapeutic target and emphasize the need for preventive strategies aimed at maintaining vascular integrity to preserve auditory function in aging and metabolically compromised populations.

SU4. Oncomodulin Attenuates Oxidative Stress-Induced Senescence in Auditory HEI-OC1 Cells

Janith Nilmika Halpage Hewa Halpage*¹, Santha Ranganathan¹, Ian Stahl¹, Dwayne Simmons¹

¹*Baylor University*

Background: Age-related hearing loss (ARHL) is characterized by the progressive dysfunction/loss of cochlear outer hair cells (OHCs), yet the molecular mechanisms contributing to their decline remain unclear. Cellular senescence, a permanent state of cell cycle arrest triggered by stress, is increasingly recognized as a contributor to age-related tissue degeneration including in the auditory system. Oncomodulin (OCM), a calcium-binding protein highly expressed in OHCs, is essential for calcium homeostasis. The hypothesis for this project is that the loss of OCM accelerates OHC senescence in auditory cells.

Methods: To investigate the potential role of OCM in regulating stress-induced senescence, we used the HEI-OC1 cochlear cell line and induced senescence using hydrogen peroxide (H₂O₂). Cells were transfected with plasmids encoding OCM (fused to GFP or mCherry). Senescence-associated β -galactosidase (SA- β -gal) and p16INK4a immunofluorescence were looked at to measure levels of senescence. To investigate the relationship between autophagy and senescence, immunofluorescence analysis was performed using antibodies against LC3B and p62.

Results: OCM-expressing cells exhibited significantly reduced senescence-associated β -galactosidase (SA- β -gal) activity compared to controls, along with decreased expression of the senescence marker p16INK4a. Morphological analysis showed increased nuclear enlargement, a characteristic feature of senescent cells, in control cells compared to OCM-transfected cells. Further investigation revealed that OCM modulates autophagic signaling. Immunofluorescence analysis of LC3B and p62 puncta demonstrated decreased autophagic stress in OCM-expressing cells, suggesting reduced autophagic flux. Additionally, gene expression analysis showed downregulation of p53, key regulator of the senescence pathway. Notably, mitochondrial DNA expression level was elevated in OCM-transfected cells, indicating enhanced mitochondrial integrity and resilience to oxidative stress.

Conclusions: These findings suggest that OCM suppresses cellular senescence in cochlear cells through the preservation of autophagic function and maintenance of mitochondrial health. This work identifies increased calcium buffering as a novel protective factor and highlights its potential relevance in mitigating ARHL.

SU5. Impact of Auditory Training on Cortical Neural Tracking of Rapid Speech

Ciaran Stone*¹, Ebtesam Sajjadi¹, Samira Anderson²

¹*University of Maryland, College Park*, ²*University of Maryland*

Background: Age-related hearing loss, and aging itself, can make understanding rapid speech challenging, as degraded processing in the auditory system limits the ability to process temporal cues. Current research suggests that these difficulties are linked to changes in the brain's temporal dynamics, including delayed and exaggerated responses to speech, which may represent compensatory mechanisms that often fail to fully restore speech perception. This

study's main goal is to explore whether auditory training with time-compressed speech can improve both behavioral performance and cortical processing, and if these improvements can be observed across different hierarchical levels of neural representation.

Methods: In this study, 94 participants were recruited: a younger normal-hearing (YNH) group, an older normal-hearing (ONH) group, and an older hearing-impaired (OHI) group. Each participant underwent pre- and post-training sessions. During these sessions, a behavioral assessment was conducted to determine each participant's threshold for 50% keyword recognition of time-compressed speech. Electroencephalography (EEG) was used to record cortical envelope tracking of continuous speech of audiobooks in both unprocessed and time-compressed conditions. All participants completed six adaptive training sessions where the time-compression rate was adjusted based on their performance.

Results: Our findings showed a significant improvement in behavioral measures after training. Both the ONH and OHI groups demonstrated better performance at the post-test. Notably, while the younger listeners still had the highest overall performance, the post-training thresholds for the ONH group approached the pre-training levels of the YNH group. These results suggest that this targeted training can partially restore rapid speech recognition, especially in older adults with normal hearing. To further understand the neural basis of these improvements, the cortical EEG data will be analyzed using a Temporal Response Function (TRF) framework to evaluate changes in neural encoding at various levels, from sensory (e.g., acoustic features) to linguistic (e.g., phonemes, words, phrases, and sentence onsets). We will present how the exaggerated cortical tracking responses previously observed in older adults change with training.

Conclusions: Our findings provide new and important insights into the potential for auditory training to improve rapid speech perception in older adults, and perhaps for their speech perception in general. The behavioral results suggest a partial restoration of function, which has impactful clinical relevance for aging adults. This type of targeted training could provide a valuable tool to help individuals overcome challenges with speech comprehension in everyday situations. Future research may investigate the long-term effects of such training and its potential application in home and clinical settings. A key takeaway is that auditory training can drive beneficial neuroplastic changes that improve speech recognition, especially in older adults with normal hearing. This work was supported by NIA P01AG055365.

SU6. Maturation of Cortical Auditory Evoked Potentials in Response to Tones and Noise

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Background: Brain structure and function undergo important changes during adolescence. Although the most significant neuroplastic changes happen during the first few years of life, cortical auditory evoked potentials (CAEPs) and auditory perceptual abilities do not become adult-like until early adulthood. The few studies that have investigated maturational changes in CAEPs during adolescence focused on a single type of stimulus, such as clicks, pure tones, or speech stimuli. However, given prior research indicating that children and adolescents may be more susceptible than adults to the negative effects of background noise during speech perception tasks, a comparison of CAEPs to tones vs. noises might yield valuable information

about the auditory encoding underlying these perceptual differences. Thus, the goals of this study were to examine (1) maturational changes in CAEPs to tones and noise, and (2) the relationship between CAEPs and speech recognition in noise in adolescents and young adults.

Methods: Ninety-four adolescents and young adults (10 – 23 years old) took part in this study. CAEPs were obtained in response to 400 ms long, 1000 Hz pure tones and band-pass noise, presented at 70 dB SPL via an insert earphone to the left ear. 800-1000 responses were recorded using surface electrodes (3-channel) and band-pass filtered between 1-30 Hz. Peak latencies and amplitudes (relative to 100 ms pre-stimulus baseline) were compared as a function of age. Speech perception in noise was assessed with the QuickSIN test.

Results: CAEP morphology differed in response to tones and noise, in that responses to noise stimuli had smaller N1 amplitudes and larger P2 and N2 amplitudes. From early adolescence to young adulthood, P1 and N1 latencies decreased in response to both tones and noise. In contrast, P2 latencies increased (tones only), as did N2 latencies (both tones and noise). P1 and N1 amplitudes tended to decrease from adolescence to young adulthood, while P2 and N2 amplitudes increased. Speech perception in noise improved during the course of adolescence, but this improvement did not appear to be associated with CAEP latencies and amplitudes to tones or noise.

Conclusions: There were extensive changes in CAEPs in the period between early adolescence and young adulthood. Results confirmed previous findings of continued maturation of CAEPs during adolescence and extend these findings in response to noise stimuli. CAEP responses to noise were markedly different from responses to tones, but results did not indicate a differential impact of age during adolescence. Other factors not examined in this study may be able to better predict maturational effects of adolescence on speech recognition in noise.

SU7. Cortical Representation of Attended and Ignored Acoustic Features Under Binaural Unmasking

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Background: Binaural unmasking refers to the auditory system's ability to utilize interaural differences to segregate a target sound from background noise. This mechanism has been linked to enhanced cortical encoding of the acoustic features of attended continuous speech. Interestingly, even when background noise is not the focus of attention, its acoustic features still manifest in cortical responses. These observations raise an important question: To what extent does binaural unmasking influence the cortical representation of ignored auditory input during continuous speech processing in noise?

Methods: Twenty young adults with clinically normal hearing participated in a listening task involving audiobook stories embedded in speech-shaped noise at a signal-to-noise ratio of -2 dB. To examine the effects of binaural unmasking, two listening conditions were employed: S0N0, where both speech and noise were presented in-phase across ears, and S π N0, where speech was delivered out-of-phase while noise remained in-phase. EEG recordings were collected throughout the task. After each trial, participants responded to comprehension questions and provided subjective ratings on how well they grasped the story's gist and the effort required to understand it. Cortical tracking of acoustic features was analyzed using multivariate temporal

response functions (mTRFs), which modeled cortical EEG responses from spectrotemporal features (envelope and acoustic onsets) of both the target speech and the noise.

Results: Compared to the S0N0 condition, the S π N0 condition yielded enhanced behavioral performance, including higher accuracy in story comprehension, stronger ratings for gist understanding, and reduced perceived effort during listening. At the neural level, cortical TRFs to both envelope and acoustic onsets of the target speech were stronger and occurred earlier in the S π N0 (vs. S0N0) condition. Moreover, the mTRF models showed higher predictive accuracy for the target speech in the S π N0 (vs. S0N0) condition when using combined acoustic features or acoustic onsets alone. Finally, although cortical tracking of acoustic features of the noise was observed, no significant differences in TRFs or model prediction accuracy were found between the two listening conditions.

Conclusions: These findings reveal an interaction between top-down selective attention and binaural unmasking in cortical processing of continuous speech in noise. Future work should explore this interplay in multi-talker conditions.

SU8. Electroencephalographic Correlates of Auditory Selective Attention in Hearing Loss

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Background: Current clinical practices for sensorineural hearing loss (SNHL) emphasize audibility, and provide amplification to compensate for elevated audiometric thresholds. Yet, even with amplification, many patients with SNHL struggle to understand speech in noisy environments. Although most research on SNHL focuses on sensory deficits, relatively little is known about how top-down selective attention processes may be altered in individuals with hearing loss. To investigate this, we simultaneously record 32-channel EEG and behavioral responses.

Methods: Simultaneous 32-channel EEG recordings and behavioral measurements data has so far been collected from 8 subjects with normal hearing and 14 subjects with mild to moderate hearing loss. The matrix sentence test material was used to create a speech-on-speech task where the target and masking streams were constructed using the same group of words in the same speaker's voice at 0 dB target-masker ratio. The target and masker streams are lateralized to opposite hemifields using interaural time differences and staggered in time. This design minimizes acoustic overlap and thus reduces peripheral sensory interference, allowing our measures to primarily reflect top-down attentional processes rather than peripheral deficits. We examined two EEG correlates of spatial attention, (1) lateralized alpha-band oscillations (7–15 Hz) in parietal and occipital regions which reflect endogenous attention allocation during the preparatory phase, and (2) enhanced evoked potential amplitudes which reflect how the top-down attention modulates the sensory representation under focused attention. Stimuli and target location were counterbalanced across trials.

Results: To quantify alpha lateralization, we used the multitaper technique to compute alpha band power over parieto-occipital channels, comparing power between 'attend-left' and 'attend-right' trials. Attentional modulation was quantified by computing evoked potentials to stimulus onset and comparing the amplitudes between 'attend-lead' and 'attend-lag' trials.

Preliminary results suggest reduced alpha lateralization in individuals with hearing loss and trends of lower attentional modulation of sensory stimulus.

Conclusions: These data (if trends hold) would provide evidence that top-down attention is also altered in SNHL over-and-beyond sensory deficits that are known to exist. Our ongoing work aims to expand our dataset and characterize how top-down attentional control is affected in SNHL, shedding light on the interplay between sensory deficits and higher-order processes.

SU9. Probing Invariances in Auditory Event Categorization Using Model Metamers

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Background: Real-world acoustic inputs contain rich sensory information that we parse into discrete auditory objects and categories. However, the transformation from acoustic waveforms to neural representations is complex and poorly understood, especially as neural representations are invariant across some feature changes, such as variation in the exact pitches or timing. Further, previous investigations of auditory category representations have largely explored speech, music, or tone structures, with very few studies exploring categorical representations of non-speech stimuli. Computational modeling allows researchers to explicitly probe the transformation from input waveforms into higher-level representations, with recent work suggesting that deep neural networks (DNNs) are well-suited to model human auditory processing. Here, we test whether the invariances of DNNs trained to categorize non-speech sound stimuli are aligned with the invariances of human observers.

Methods: We developed an experimental paradigm to compare humans and DNNs using forced-choice classification across 25 non-speech sound categories using sounds obtained from the FSD50K dataset. For the tested models, we generated ‘model metamers’—stimuli whose activation produces the same response as the natural stimulus at a given model stage. We then tested whether humans (n=4) could recognize the model metamers as well as they could recognize the original audio. Recognizable model metamers imply that invariances of the model are shared with those of human observers. We tested DNNs pretrained on three different tasks: (1) word recognition only, (2) auditory event recognition only, and (3) a multi-task model for word recognition, speaker recognition, and auditory event recognition. These models were trained to be robust to small “adversarial” perturbations in the input cochleagram, a technique that was previously shown to help align the invariances of models with the invariances of human observers in the speech domain. For comparison of including this type of training, we also tested a multi-task model without adversarial training.

Results: First, humans and models can reliably categorize the natural sounds, demonstrating that our novel behavioral paradigm is well suited for testing invariances in auditory categories. For all models, we found that recognition accuracy for model metamers dropped significantly, but there was variability across models. Metamers generated for the adversarially trained auditory event recognition model were the most recognizable among the four models. This result indicates that task-specific optimization plays a significant role in creating human-like invariances. Among the multi-task models, metamers from the adversarially trained model had significantly

higher accuracy, confirming that training with adversarial perturbations helps align model invariances more closely with those of humans.

Conclusions: The results indicate that model metamers provide a powerful tool for probing human auditory perception in the domain of categorical representations of auditory events. These findings provide additional evidence that adversarial training and task-specific optimization can improve model alignment with human perception.

SU10. Neuronal Correlates Underlying Auditory Categorization in Mice

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Background: Categorization of sensory stimuli is a fundamental computation in cognition, and is critical for many aspects of learning, perception, and prediction. Auditory categorization (AC) has been linked to the neuronal activity in the auditory cortex, however, it remains poorly understood how categorical representations emerge in population neuronal activity with learning.

Categorization is often influenced by prior information and experience and can be modified by new information. We recently showed that during categorization learning, mice use multiple complementary strategies (Collina et al., 2025). These strategies affect the animals' perception of stimuli category under uncertain conditions, e.g., for stimuli that surround the categorization boundary. Additionally, animals modify these strategies based on their training. To understand neuronal correlates of auditory categorization, we recorded neuronal activity from the auditory cortex of mice using implanted high-density electrodes while training them to perform an auditory categorization task.

Methods: Mice were implanted with custom-made chassis housing Neuropixels electrodes in the auditory cortex and trained to discriminate between high and low frequency tones in 2-alternate forced choice (2AFC) task. Once their behavioral performance reached criterion %, we tested how well population neuronal activity categorized ambiguous tones around the categorization boundary, and how this boundary was affected by biasing the distribution of the stimuli toward high or low frequency tones.

Results: Single neuron analysis indicated that approximately 30% of the recorded neurons exhibited category preference, as measured by the difference in the firing rate of neurons was for stimuli between categories than within categories. Additionally, population decoding accuracy to between categories stimuli increased with training. To examine how neuronal population reflect behavior, we compared the neurometric and psychometric thresholds across sessions. Training increased alignment between thresholds of neurometric and psychometric curves with well aligned threshold estimates in fully trained animals. Moreover, a subpopulation of neurons was choice-selective, as their firing rate reflected the choice of the mouse across trials. Additionally, we also identified components in the population responses that signaled reward prediction, reward response and reward prediction error in AC.

Conclusions: Our results show that neuronal activity in the auditory cortex during auditory categorization reflects behavioral performance. We are currently analyzing how the neuronal

population activity evolves throughout auditory learning and how biasing the category distribution across different sessions affects neuronal activity.

SU11. Reduced Sound Tolerance due to Enhanced Central Gain in a Rat Model of Fragile X Syndrome

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Background: Atypical sound processing is one of the most common and quality-of-life affecting symptoms seen in autism spectrum disorders (ASD). Fragile X Syndrome (FXS) is the leading inherited cause of ASD and a majority of FXS individuals present with auditory processing issues. Despite this prevalence and centrality to the autistic phenotype, relatively little is known about the nature and mechanisms of auditory perceptual impairments in FXS and ASD.

Methods: We characterized auditory processing in adult male Fmr1 KO rats and wildtype littermates using a comprehensive battery of behavioral tasks that take advantage of the highly trainable nature of rats and their innate capacity to express complex behavior. We combined these behavioral assays with multi-region electrophysiological recordings and computational modeling to determine the neural origins of atypical sound-driven behavior in these animals.

Results: We found that Fmr1 KO rats exhibited heightened perceptual sensitivity and impaired gating of incoming auditory information, resulting in reduced sound tolerance and exaggerated avoidance behavior. Despite this behavioral evidence for sound hypersensitivity, Fmr1 KO rats exhibited diminished suprathreshold sound-evoked responses at the level of the auditory nerve and brainstem. However, this reduced afferent drive was progressively amplified along the ascending auditory pathway, culminating in several cortical network alterations that could account for observed perceptual differences, including increased spontaneous firing rates, steeper sound intensity-response functions, and broader frequency tuning. Using an experimentally informed Bayesian population decoder, we demonstrated that we could recapitulate behavioral deficits from auditory cortical but not subcortical neurophysiological recordings.

Conclusions: These findings are reminiscent of central gain changes observed along the central auditory pathway in response to hearing loss, suggesting a potential shared etiology for hyperacusis of diverse origin. Moreover, our results indicate that cortical hyperexcitability may account for a range of auditory behavioral phenotypes in FXS, providing a potential locus for development of novel biomarkers and treatment strategies that could extend to other forms of ASD.

SU12. Somatostatin Interneurons Drive Contrast Adaptation in the Mouse Primary Auditory Cortex

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Background: In natural environments, behaviorally-relevant sounds such as speech often co-occur with background noise such as wind or traffic. In order to effectively identify and respond to relevant sounds, the auditory system must isolate signal from noise. This ability is reduced in

people with hearing impairments and auditory processing disorders, posing unique challenges in navigating everyday environments. In order to develop effective treatments, it is vital to understand the mechanisms underlying hearing in noise.

One strategy for isolating signal from noise is to adapt to the current auditory environment. In the primary auditory cortex (A1), neurons adapt to changes in stimulus variability (contrast) by adjusting their gain. This process is known as contrast gain control (CGC). Though CGC has been demonstrated across many organisms from mice to humans, its mechanisms are still unknown.

In this study, I test the hypothesis that two different classes of inhibitory interneurons, parvalbumin (PV)- and somatostatin (SST)-positive interneurons, drive contrast gain control in mouse A1.

Methods: In order to test the role of different inhibitory interneuron subtypes in driving CGC, we optogenetically suppress PV or SST populations in A1 of mice presented with stimuli of changing contrasts. We test for CGC using two methods: behavioral performance on a target-detection task, and extracellular electrophysiological recordings in A1.

First, we tested how optogenetically suppressing different populations of interneurons affected behavioral performance on a target-detection task. Mice were presented with stimuli of low and high contrasts and trained to respond when they detect an increase in sound pressure level. Previous experiments in mice and humans show that performance on similar tasks increases in low-contrast backgrounds, corresponding to increased neuronal gain. If PV or SST neurons drive CGC, we expect optogenetic suppression of these populations to reduce the change in performance between the two contrasts.

Second, we performed acute extracellular recordings in mice passively exposed to auditory stimuli with high and low contrasts. Using these neuronal responses, we can directly calculate neuronal gain changes across stimulus contrasts. If PV or SST neurons drive CGC, we expect optogenetic suppression to reduce the degree of gain adaptation between the two contrasts.

Results: We find that SST suppression, but not PV suppression, impairs CGC between high and low contrast conditions. Though analysis of the extracellular data is still in progress, we observe a similar trend of decreased CGC during SST but not PV suppression. These results suggest that SST-positive interneurons drive CGC in mouse A1.

Conclusions: This research finds that SST neurons are involved in driving contrast adaptation in the cortex. This opens up new avenues of research into the potential role of SST neurons in auditory processing disorders.

SU13. Multiscale Adaptation Dynamics Reflect Hierarchical Predictive Processing in the Ferret Auditory Cortex

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Background: The mammalian brain encodes regularities with adaptation across multiple timescales: from rapid stimulus-specific adaptations in short-scale, to predictive processing of complex sequence structures. Previous studies, across species, suggest a cortical gradient of integration windows, from A1 to higher auditory and supramodal areas, but details of both their flow and temporal generalization remain unknown. Our goal is to dissociate responses to omission, local, and global violations in ferret A1 and PEG and to quantify their shared information and integration depth.

Methods: We recorded 32-channel electrocorticography (ECoG) covering the primary auditory cortex (A1) and the posterior ectosylvian gyrus (PEG) in ferrets, while presenting hierarchical sequences of pure tones or natural syllables. We used the local-global oddball structure as the main framework for our experiment design, and the sequences with various lengths comprised a repetition stage followed by a final event that either conformed to, violated, or was omitted in relation to the learned temporal pattern.

Across experiments, we manipulated: (i) final-item change after repetition (local violation), (ii) omission at sequence end, and (iii) higher-order pattern changes across different trials of the experiment run (global violation), including cross-item substitutions and re-ordering. Analyses combined condition-wise ERPs, time-resolved linear decoding, and temporal generalization matrices within and across areas. Finally, a dynamic Bayesian observer estimated per-channel integration depth by fitting trial-wise neural “surprise” derived from the event-locked mismatch windows.

Results: Local violations expressed an early MMN-like offset-locked response (< 200 ms) with strong shared information between A1 and PEG. Linear decoders indicated a stronger A1 contribution at this stage. A later component (≈ 250 – 500 ms) was more temporally sustained in PEG, showed broader temporal generalization within PEG compared to A1, and reduced cross-area decodability between A1 and PEG, representing area-specific updatings. Global violations emerged later (≈ 450 – 700 ms), partially dependent on the presence and magnitude of the earlier local response, and appeared as short sets of pulse-like signals in temporal generalization rather than sustained, time-invariant codes.

The Bayesian observer showed predictions consistent with the general pattern of our recorded surprisals. The model used to estimate the best-fitting integration depths and compare them in A1 and PEG. These effects are observable in tone sequences to syllabic sequences with different lengths and stimulus structures.

Conclusions: The results are consistent with hierarchical predictive processing with parallel mechanisms: fast, MMN-like, shared prediction errors tied to terminal-event changes are strongly expressed in both A1 and PEG, followed by longer-window, field-specific positive ERPs that are more prominent in PEG for the local deviation. We also observed meaningful responses for omission and global effects. Model-based estimations can investigate the scale of effective integration windows in each area. Together, these results quantify how cortical fields coordinate prediction errors and updates across timescales, linking computational prediction of surprise to mesoscale physiology.

SU14. Binaural Interaction Creates Diverse Azimuth Tuning Curve Shapes Across Auditory Midbrain Neurons

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Background: Neurons in the central nucleus of the inferior colliculus (ICC) exhibit spatial receptive fields due to underlying neural sensitivity to acoustic cues that covary with sound source location, including binaural cues (interaural time difference [ITD] and interaural level difference [ILD]), and monaural cues (spectral shape and acoustic gain within each ear). The present study examines how these cues shape each neuron's azimuth tuning curve (i.e., the function of firing rate vs. source location within the front horizontal plane), and whether the effects of cues differed with neural characteristic frequency (CF).

Methods: Broadband noise stimuli were presented to awake rabbits of both sexes in virtual acoustic space using each rabbit's own head-related transfer functions. Stimuli were presented either monaurally (contralateral ear) or binaurally, with transfer functions digitally manipulated in some conditions to isolate neural sensitivity to ITD or ILD.

Results: For low-CF neurons (< 2.8 kHz), azimuth tuning curves measured under monaural presentation were mostly flat, whereas curves measured under binaural presentation took on a diversity of shapes, such as functions sloping upwards or downwards from ipsilateral to contralateral azimuths; functions with broad, bell-shaped tuning to various azimuths; and functions with complex shapes. Binaural interaction (i.e., the effect on firing rate of two ears vs. contralateral ear only) caused facilitation of rates in most low-CF neurons; rate facilitation or suppression was not biased to particular azimuths and could be driven by neural sensitivity to ITD, ILD, or the covariation of both. Unlike low-CF neurons, high-CF neurons (> 2.8 kHz) had azimuth tuning curves measured under monaural presentation that took on a diversity of shapes; binaural interaction in these neurons changed the shapes of tuning curves, but the shapes remained diverse. Binaural interaction caused suppression of rates in most high-CF neurons, which was biased towards ipsilateral azimuths; however, rate facilitation found in a minority of neurons was not biased to particular azimuths. Unlike low-CF neurons, rate suppression or facilitation in high-CF neurons was overwhelmingly driven by neural sensitivity to ILD.

Conclusions: Consistent with a diversity of tuning curve shapes found across both low- and high-CF neurons, the distribution of firing rates over the neural sample evoked by the noise stimulus was constant across azimuths except for a small decrease at the most extreme ipsilateral locations (60°–90°). Therefore, the pattern of firing rate activity across ICC neurons was inconsistent with azimuth decoding models that rely upon summed population activity.

SU15. Aging and Synaptopathy Lead to Impaired Temporal and Binaural Processing in the Auditory Brainstem, and Spatial Hearing, in the Mongolian Gerbil

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Background: Aging is known to lead to difficulty in binaural and spatial hearing, often before affecting hearing thresholds. One potential source of this may be Cochlear Synaptopathy (CS), or the loss of synapses between inner hair cells and auditory nerve fibers. CS occurs during aging, and is present prior to raised hearing thresholds. The mechanisms of how age-related CS could disrupt binaural hearing are unclear, but they likely involve the auditory brainstem, the first site to receive input from the auditory nerve fibers and the earliest site for binaural processing. Here, we assess behavioral, physiological, and histological measures of auditory brainstem dysfunction from aging and synaptopathy in the Mongolian Gerbil (*Meriones unguiculatus*).

Methods: Cohorts consisted of young (2-10 month) and aged (> 30 month) gerbils. We recorded auditory brainstem responses (ABRs), envelope following responses (EFRs), spatial hearing behavior, and in vivo single-cell electrophysiological responses from cochlear nucleus and trapezoid body units. From the ABRs, we also measured the Binaural Interaction Component (BIC), a biomarker of spatial hearing abilities. To see if these measurements correlated with behavioral changes, gerbils were tested utilizing prepulse inhibition of the acoustic startle response (PPI) on two sets of tasks: 1) gap detection in broadband noise and 2) sound location spatial acuity. We also assessed synaptopathy by staining cochlea for presynaptic (Ctbp2) and postsynaptic (GluA2) markers and inner hair cell bodies (Myosin VIIa).

Results: We found an increase of synaptopathy, quantified by a decrease of colocalized pre- and postsynaptic markers per inner hair cell, in aged gerbils compared to the young cohort. This corresponded with a decrease in ABR Wave I and Wave III amplitudes, but not a significant reduction in hearing thresholds. The impaired ABRs and lack of threshold shifts indicate an impairment of neural synchrony in the brainstem. Aged animals exhibited reduced BIC amplitude, as well as impaired modulation of BIC amplitude with variation of interaural time differences (ITDs), indicating a reduction of brainstem binaural encoding in aged animals. We also found a reduced synchrony in EFR recordings, further showing physiological dysfunction in the auditory brainstems of the aged cohort. These findings corresponded with a reduction of synchrony to amplitude modulated stimuli recorded in vivo from bushy cell axons and medial nucleus of the trapezoid body (MNTB) neurons. Behaviorally, aged animals had higher thresholds of detection for both gap length and spatial acuity, indicating impaired temporal and spatial hearing behaviors.

Conclusions: Collectively, these data reveal that aged gerbils have impaired auditory brainstem physiology, partly as a result of synaptopathy, which underlies behavioral issues in temporal and spatial auditory processing similar to that exhibited by aged humans. [Supported by NIDCD 1F31DC021622-01]

SU16. Impaired Temporal Precision in Auditory Brainstem Processing Underlies Sound Localization Deficits in a Novel Mouse Model of Spinocerebellar Ataxia Type 13

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Background: Spinocerebellar Ataxia Type 13 (SCA13) is an inherited motor disorder characterized by an additional deficit in sound localization, despite otherwise normal hearing. This deficit arises from point mutations in the gene encoding the voltage-gated potassium channel Kv3.3, which is essential for neurons that support high-frequency firing, such as those in the auditory brainstem. To investigate the neural circuit mechanisms underlying this auditory impairment, we developed a novel CRISPR-Cas9 mouse model expressing the human R420H Kv3.3 mutation, which disrupts the S4 voltage sensor and impairs channel function.

Methods: The investigation was conducted using whole-cell patch-clamping, in vivo single unit recordings and imaging using immunostaining.

Results: Histological analysis of the auditory brainstem in R420H mutant mice revealed only a mild, age-unrelated reduction in cell numbers within the Lateral Superior Olive (LSO), without evidence of significant neurodegeneration. This suggests that the observed deficits are primarily functional rather than due to degenerative cell loss. A slight disruption in Kv3.3 protein transport was also noted, which might further contribute to the phenotype. However, the most pronounced effects of the mutation were observed in action potential (AP) dynamics.

Whole-cell patch-clamp recordings from brainstem slices demonstrated that the R420H mutation substantially broadens APs, particularly in the LSO (with a 2–3-fold increase in AP half-width) and to a lesser extent in the medial nucleus of the trapezoid body (MNTB; 1.5–2-fold increase). As a result, both MNTB and LSO neurons showed high failure rates during high-frequency stimulation (500 Hz in MNTB and 200 Hz in LSO). In the LSO, firing failures were strongly correlated with prolonged AP duration and were further exacerbated by significantly extended excitatory and inhibitory postsynaptic currents.

In vivo single-unit recordings confirmed that broadened APs reduce the maximum firing rates of MNTB and LSO neurons. Importantly, we found a marked imbalance between ipsilateral excitation and contralateral inhibition converging onto LSO principal cells. This disruption in synaptic integration manifested as a significantly reduced binaural interaction component (BIC) in auditory brainstem response (ABR) recordings.

Conclusions: Collectively, these results demonstrate that the R420H Kv3.3 mutation compromises the temporal fidelity of auditory brainstem neurons, disrupting the precise balance of excitation and inhibition necessary for accurate sound localization. This establishes a direct mechanistic link between ion channel dysfunction, neural circuit impairment, and the specific auditory deficits observed in SCA-13.

SU17. Active Sensorimotor Strategies for Sound Localization in Complex Acoustic Scenes

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Background: In natural environments, animals must react appropriately to behaviorally relevant sounds, such as those signaling predators or food sources, while ignoring competing auditory signals. Effective responses require identifying both the identity and location of sounds. The psychophysics of sound localization have been well characterized by presenting stimuli at varying positions relative to an animal's head, which is kept stationary for precise spatial control.

However, such approaches do not capture the sensorimotor strategies animals use during active navigation. In real-world settings, auditory spatial perception is shaped by environmental noise, behavioral context, and self-motion. Animals frequently orient their heads and bodies to reduce perceptual ambiguity and improve localization accuracy. To investigate the behavioral strategies that support naturalistic sound localization, we developed a freely-moving auditory discrimination task.

Methods: We trained C57BL/6J mice to localize a target sound along the perimeter of an open, circular arena while ignoring a distractor presented simultaneously from another azimuthal position. Stimuli were bandpassed white noise of varying center frequencies. After trial initiation, both stimuli played continuously until mice approached and licked a water spout beneath the perceived target. Since mice were free to explore before making a decision, this design enabled observation of how animals integrate dynamic sensory input with self-motion.

Results: Over several weeks of training, mice adopted characteristic sensorimotor strategies that facilitated task learning, including rapid head and body “scanning” movements at stimulus onset and 180° turns to correct errant approaches towards the distractor. These behaviors became more frequent with learning and coincided with increased accuracy, shorter reaction times, and more stereotyped trajectories.

Conclusions: These results demonstrate that in an unconstrained sound localization task, mice resolve spatial ambiguity with dynamic movement strategies and update motor plans continuously in response to ongoing acoustic input. Our ongoing work investigates the neural circuits underlying this dynamic sensorimotor loop. While auditory cortex encodes spectral features and learned associations, it lacks a map of egocentric space. In contrast, its projection targets in the midbrain, such as the superior and inferior colliculi, are spatially tuned and mediate orienting behaviors. We are currently using pharmacological and optogenetic perturbations to dissect how corticocollicular circuits contribute to spatial decision-making, offering new insight into how animals transform auditory input into adaptive action.

SU18. The Impact of Response Method on Sound Localization Performance

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Background: Sound localization is a key marker of binaural hearing, relying on the brain’s integration of interaural timing and level cues. Despite its clinical relevance, conventional localization assessments lack ecological validity and do not capture the reflexive nature of natural orienting. Standard methods, such as pointing or reporting a speaker number, require conscious decision-making and are susceptible to visual biases, potentially limiting their sensitivity to true auditory spatial perception. In natural listening, orienting to a sound involves a coordinated sequence of eye and head movements: the eyes typically move first, providing a rapid, low-latency estimate of target location, followed by slower head adjustments that align auditory and visual cues. We hypothesize that combined reflexive head-and-eye orienting offers a more direct and automatic measure of spatial hearing. This study examines how response modality influences localization performance in normal-hearing adults, with attention to both accuracy and precision.

Methods: Fifteen normal-hearing adults participated in a within-subject, repeated-measures design. Localization was assessed under two hearing conditions: normal binaural hearing and simulated monaural hearing (earplug + muff). Broadband Gaussian white noise (0.2–20 kHz) was presented at 50, 60, or 70 dBA from 19 loudspeakers spanning $\pm 90^\circ$ in azimuth, separated by 10° . Tasks were performed in both vision-allowed and vision-denied conditions to examine the influence of visual cues while eliminating feedback during trials. Participants completed three response modalities: (1) head movement alone, (2) combined head and eye tracking, and (3) verbal reporting of speaker number (vision only). For each trial, head and eye trajectories, reaction times, and response times were recorded. Localization performance was quantified using regression of target azimuth onto participant responses, providing gain, bias, and R^2 . Mean absolute error measured overall accuracy, while trial-to-trial variability captured precision.

Results: Response modality significantly influenced localization outcomes. Combined head-and-eye tracking demonstrated lower mean absolute error and reduced variability compared with head-only responses. Verbal reporting was associated with longer reaction times and higher variability, reflecting additional cognitive and motor demands. Simulated monaural hearing amplifies these differences, with reflexive orienting responses maintaining greater sensitivity to spatial cues than verbal responses. Regression analyses suggests improved alignment for combined head and eye tracking with true target locations.

Conclusions: Response method shapes both the accuracy and temporal dynamics of measured localization. These results suggest that reflexive orienting responses, particularly combined head and eye movements, provide a more precise and sensitive measure of auditory spatial perception than traditional methods. These findings underscore the importance of ecologically valid response modalities in both experimental and clinical assessments of spatial hearing. Careful consideration of response method has implications for evaluating auditory performance, designing experiments, and interpreting outcomes in hearing-impaired populations or users of hearing-assistive devices.

SU19. The Impact of Chloride Homeostasis on the Development of LSO Neurons

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Background: In the developing nervous system, inhibitory inputs are often transiently depolarizing due to high intracellular chloride concentrations, a process thought to guide neuronal maturation and synaptic refinement. In the auditory brainstem, this chloride gradient is primarily regulated by the transporters NKCC1 and KCC2. However, the specific role of this early depolarizing inhibition in shaping developing auditory circuits remains unclear.

Methods: To investigate the functional impact of early depolarizing inhibition, we used a mouse line with a double mutation in the KCC2 gene that enhances chloride extrusion and abolishes depolarizing GABA/glycinergic responses. We performed whole-cell patch-clamp recordings in acute brainstem slices targeting principal neurons of the lateral superior olive (LSO), a nucleus with well-characterized inhibitory inputs. Experiments were performed in animals at postnatal day 14 (P14) and P20, corresponding to the period shortly after hearing onset and following early auditory experience.

Results: We found that gross morphological development and intrinsic membrane properties of LSO neurons in KCC2 mutant mice were comparable to those in wildtype animals. However, afferent fiber stimulation revealed that amplitudes of inhibitory postsynaptic current (IPSC) amplitudes were significantly reduced with no change in IPSC kinetics or short-term plasticity. A similar reduction in current amplitude was observed with direct application of glycine, suggesting that the decrease in IPSC size is due to postsynaptic alterations such as a reduction of glycine receptors.

Conclusions: Our preliminary findings suggest that while early depolarizing inhibition is not essential for gross morphological development or maturation of intrinsic membrane properties of LSO neurons, it might be critical for fine-tuning synaptic strength, likely through a postsynaptic mechanism involving the regulating glycine receptor expression.

SU20. Maturation of Intrinsic Membrane Properties in Octopus Cells of the Mouse Cochlear Nucleus

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Background: During postnatal development, synaptic refinement and electrophysiological maturation occur simultaneously to finely tune auditory brainstem circuits. Before ear canal opening, spontaneous peripheral activity establishes precise circuitry across the central auditory system. When sound-driven activity begins, further refinement occurs through synaptic pruning. For octopus cells of the mammalian ventral cochlear nucleus, extreme intrinsic membrane properties are essential for the submillisecond temporal integration that supports spectral edge detection. Octopus cells in mature mice have low input resistances ($\sim 4\text{--}10\text{M}\Omega$), fast time constants ($\sim 200\mu\text{s}$), and large low-voltage-activated potassium and hyperpolarization-activated conductances ($\sim 40\text{nS}$ and $\sim 60\text{nS}$ at rest, respectively). These membrane properties, combined with the large diameter dendrites, allow for rapid and precise integration of auditory nerve input. We aim to define when these anatomical and physiological specializations emerge relative to the onset of hearing.

Methods: We examined the timeline of octopus cell maturation using in vitro whole-cell current clamp recordings from postnatal day 4 to 35 (P4–35) mice. Active and passive membrane properties including input resistance, time constant, HCN sag ratio, and action potential half-width were assessed. Auditory nerve inputs were evoked by electrical stimulation, and cells were filled with biocytin for post hoc three-dimensional reconstruction of dendritic arbors.

Results: By the time of ear canal opening (P10–12), intrinsic properties of octopus cells are relatively mature. Measures of input resistance, time constant, HCN sag ratio, and spike half-width are comparable at P10 and P16. Further, reconstructions of dendritic arbors revealed no meaningful changes between P10 and P16. Despite this stability at the onset of hearing, octopus cells underwent a rapid transformation between P4 and P10. During this early period, dominated by spontaneous cochlear activity, input resistance decreased, time constant shortened, sag ratio increased, and action potentials narrowed.

Conclusions: The timeline for anatomical and physiological development suggests that the properties underlying octopus cell function are established by the time sound-driven activity begins. Octopus cells rapidly mature when spontaneous waves of activity from the cochlea

dominate activity patterns in the octopus cell circuit. The rapid maturation that precedes ear canal opening highlights a critical developmental window shaped by spontaneous activity rather than acoustic experience. Octopus cells thus enter the onset of hearing configured for fast temporal integration. Future work will test how altering peripheral activity during this early window influences octopus cell development. By comparing normal and disrupted activity patterns, these experiments will define the contribution of spontaneous cochlear activity to the emergence of octopus cell function.

SU21. The Effect of Electrode Placement on the Auditory Brainstem Response

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Background: The auditory brainstem response (ABR) is commonly used both in the clinic and the lab to estimate hearing thresholds and examine the subcortical auditory system. However, the location of the neural generators and the size of the responses lead to poor signal-to-noise ratio (SNR), requiring long test times to acquire an adequate signal. Typically, earlobes or mastoids are used as the reference electrodes, but other reference locations may improve signal quality and speed response acquisition. Previous work has shown that the nape of the neck reduces muscle artifact in subcortically generated frequency-following responses, which may also benefit the ABR.

Methods: Subjects were presented with one hour of randomly timed click stimuli at 80 dB peSPL with a mean rate of 40 clicks per second. EEG was recorded using a Biosemi Active3 system with a 64-channel cap, and additional electrodes were placed on the earlobes, nose, nape of the neck, and collarbone for offline re-referencing during analysis. Two more electrodes were placed by the left eye to aid in the removal of eye-related artifacts.

Responses were computed separately for the left ear and right ear clicks. The SNRs of the responses were estimated based on the variance of the waveform in the response window compared to the baseline variance. For each reference electrode, responses were re-referenced and the SNR was calculated for the response from each electrode in the cap to identify optimal placement of electrodes to obtain the ABR.

Results: Preliminary results indicate the nape and earlobes to be the best choices for reference electrodes, producing responses with the highest SNRs. The nape provides a larger ABR wave V and less muscle artifact than earlobes. The combined earlobes had the next best SNR. Wave I was most visible when referenced against the ipsilateral earlobe. The positive electrodes with the best SNRs were located near the vertex of the head for all references, with commonly used frontal electrodes showing worse SNR.

Conclusions: The specific electrode montage can have a large impact on the SNR of ABRs. In a bipolar montage, the location of both electrodes should be considered in the context of the specific goals of the recording (e.g. fast overall response detection versus examination of wave I). Selecting the optimal montage may yield substantial improvements in response quality, which is especially important when recording time is limited.

SU22. Dopamine Receptor Expression in the Mouse Superior Olivary Complex

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Background: Neuromodulatory circuits influence auditory neural computations by regulating presynaptic and/or postsynaptic mechanisms at multiple levels of processing. However, modulation in auditory regions between the periphery and the cortex have remained largely unexplored. Recent work by our laboratory has demonstrated several mechanisms of modulation in the superior olivary complex (SOC) by a network of cholinergic inputs (Beebe et al. 2021, Zhang and Burger 2024, Weimann et al. 2024). In addition to cholinergic input, dopaminergic projections to the SOC have been documented (Nevue et al. 2016). However, there have been no studies demonstrating expression of dopaminergic receptors or physiological modulation by dopamine in SOC neurons. While dopaminergic neurons compose a small population in the brain, they have potent and widespread impacts on targets throughout the nervous system. A diverse array dopamine receptor subtypes (D1-5), underlie the variable physiological outcomes of dopaminergic modulation. Dopamine receptors are members of the G protein-coupled receptor family and divided into two classes. D1 and D5 belong to the D1-like family while D2, 3, and 4 are members of the D2-like family. Each of these families of dopamine receptor subtypes initiate unique downstream cascades.

Methods: To investigate the potential auditory processing function(s) of the dopaminergic input to the SOC, we first used immunohistochemistry to describe the expression patterns of dopamine receptor subtypes 1-5 in mouse SOC neurons in mice aged P10 and P30 before and after hearing onset (~P12). Antibodies directed against D1-D5 receptors were used to examine expression in the principal nuclei of the SOC. Presynaptic expression was confirmed by colocalization of dopamine receptor stain with VGLUT1.

Results: We demonstrate that all five DA receptor subtypes are differentially expressed in the mouse SOC. D1 is expressed in SOC nuclei pre and post hearing onset. In the MNTB, D1 appears to have weak expression early in development with strong post-synaptic expression later at P30. D2 appears to be both pre and postsynaptically expressed at P10 with primarily postsynaptic expression by P30. D3 demonstrates a primarily presynaptic expression pattern across development in the MNTB and the LSO. D4 exhibits presynaptic expression at P10 with stronger postsynaptic expression by P30 in the MNTB while the LSO exhibits primarily postsynaptic expression. Finally, our staining suggests that D5 receptors are weakly expressed across development in SOC nuclei.

Conclusions: Our results show for the first time that DA receptor subtypes 1-5 are expressed in the mouse SOC with unique expression profiles. These results strongly suggest that dopamine is likely to provide neuromodulatory signaling to the SOC.

SU23. Age-Dependent Regulation of Calcium Responses in the Mouse Medial Nucleus of the Trapezoid Body Neurons

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Background: In the central auditory system, Ca²⁺ signaling plays a pivotal role in the regulation of cellular processes including neuronal development and maturation. Disruption of Ca²⁺ homeostasis and signaling causes abnormal development of auditory circuits, compromising auditory processing and behavior. Here, using Ca²⁺ imaging in brain slices obtained from mice that genetically expressed calcium indicator GCaMP in glycinergic cells, we investigated the underlying mechanisms for developmental Ca²⁺ regulation in neurons of the medial nucleus of the trapezoid body (MNTB), a brainstem nucleus involved in sound localization.

Methods: Brainstem slices with visually identified MNTB were prepared from GlyT2-Cre/tdT-GCaMP6f mice of either sex, which is a transgenic line obtained by crossing the glycine transporter 2-Cre recombinase mouse to the reporter line ROSA26-tdT-GCaMP6f. Ca²⁺ imaging experiments were performed at several milestone ages of development including P0, P7, P14 and P21, at 35 °C. Fluorescence images of principal MNTB neurons were obtained under excitation wavelength of 480 nm. Ca²⁺ signals were recorded in response to activation of various excitatory and inhibitory transmitter receptors through bath application of their respective agonists and under electrical stimulation applied to their afferent inputs.

Results: Prior to hearing onset at postnatal day 7 (P7), Ca²⁺ signals were produced in MNTB neurons when the glutamate receptors (NMDARs, AMPARs, group I mGluRs) were activated by bath application of their respective agonists (NMDA 200 μM, AMPA 100 μM, 3,5-DHPG 200 μM). After hearing onset (P14 and P21), the responses declined dramatically for NMDA receptors and group I mGluRs. AMPAR-mediated signals remained relatively high. In contrast, Ca²⁺ transients in response to GABA (100 μM) and glycine (200 μM) were only observed in neonatal mice and diminished a few days after birth. Transmitter receptor-mediated Ca²⁺ signaling was largely blocked by their specific antagonists. Furthermore, electrical stimulations applied to the excitatory afferent fibers innervating MNTB, triggered release of glutamate which elicited Ca²⁺ signals, in a stimulus intensity- and frequency-dependent manner, via glutamate receptors and potentially voltage-gated Ca²⁺ channels. Although, cells from all experimental age groups were able to generate action potentials with no significant differences, under whole cell patch-clamp recordings, using similar stimulation parameters. However, calcium signals elicited in response to electrical stimulations, followed similar down-regulation fashion as the drug applications, in the MNTB neurons, over the ages of development.

Conclusions: Taken together, Ca²⁺ signaling in MNTB neurons, mediated by various transmitter receptors was developmentally down-regulated and subject to refinement by hearing experience.

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SU24. Effects of Cochlear Synaptopathy on Single-Unit Cochlear Nucleus Responses in the Budgerigar

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Background: Cochlear synaptopathy (CS) is a commonly occurring pathology in aging humans, defined as the loss of synapses between hair cells and auditory-nerve fibers (ANFs). CS is hypothesized to impair perception of sounds in noise, but underlying neural mechanisms are unknown. Recent studies suggest that CS can amplify auditory-nerve onset responses and alter temporal dynamics. However, effects of CS on brainstem encoding remain less explored. The current study quantified effects of CS on neural encoding in the nucleus angularis (NA) of the budgerigar, a parakeet species. NA is one of two avian brainstem cochlear nuclei receiving direct input from the auditory nerve. Budgerigars were selected based on recent studies of kainate-induced CS in this species and previous behavioral research demonstrating performance comparable to humans for various complex auditory discrimination tasks.

Methods: The current experiment compared basic NA response properties including frequency tuning curves, post-stimulus time histograms (PSTHs), and rate-level functions between control and CS-induced budgerigars. In CS animals, substantial (60-70%) ANF loss was induced via intracochlear infusions of 1-2 mM kainic acid. Distortion-product otoacoustic emissions were used to confirm normal hair-cell function. For in-vivo recordings of NA units, a craniotomy was made above the anterior, vertical semicircular canal. A glass electrode with 40 to 80 M Ω impedance was placed in the flocculus of the cerebellum and driven into NA to record single-unit responses. PSTHs were obtained with a characteristic-frequency (CF) tone presented 30 dB above threshold. Rate-level functions were created by randomly presenting CF tones of varying sound levels (0 to 80 dB SPL).

Results: NA neurons in control budgerigars had primary-like, onset, and chopping PSTHs in response to CF tones. Tuning curves were V-shaped, with characteristic frequencies within the range of the budgerigar's behavioral sensitivity (i.e., up to 5-6 kHz). Strength of synchrony was calculated from PSTHs using vector strength (VS). VS was greatest below 1 kHz and generally insignificant above 2 kHz, in contrast to budgerigar ANFs which show significant VS up to 4-5 kHz. From rate-level functions, we found that some NA units monotonically increased firing rate as sound levels increase, while others had nonmonotonic rate-level functions. NA units in CS-induced animals showed the same PSTH response categories and had broadly similar tuning curves, VS of phase-locking, and rate-level functions to control units. Population results are compared between groups to quantify changes in NA encoding with CS.

Conclusions: Preliminary results suggest that NA units in the CS-induced budgerigars have similar characteristics to control units. Further analyses will test population differences and characterize potential changes in onset activity, adaptation, and envelope encoding in CS-induced animals. Research in this species can provide important insight into the neural bases of normal and hidden hearing loss.

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SU25. Semilunar Nucleus: Physiology, Connections, and mGluR Modulation

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Background: Besides the calyx of Held input, additional non-calyceal inputs to the medial nucleus of the trapezoid body (MNTB) have been reported, but their functions and origins are largely unknown. Recently, a non-calyceal input originating from the semilunar nucleus (SLN) to the ipsilateral MNTB was identified in the rat (Gómez-Martínez et al. 2023, Front Neuroanat). To understand the function of this novel neural circuit, we investigated the physiology and connectivity of SLN in mice, with a focus on neuromodulation mediated by group I metabotropic glutamate receptors (mGluR1 and mGluR5).

Methods: We used a combination of brain slice electrophysiology, immunohistochemistry, and viral tracing. We conducted whole-cell patch clamp and cell-attached recordings at 35 °C in brainstem slices from mice of two age groups (< HO, before hearing onset, P7-P12; > HO, after hearing onset P13-P39). The SLN was identified by anatomical landmarks and/or calbindin1 expression pattern. Immunohistochemistry was used to examine the expression of mGluR1, mGluR5, and calbindin1. Retrograde viral tracing was used to reveal connections between SLN and other auditory structures. Mean and number of cells are reported.

Results: SLN neurons were highly excitable, with a low current threshold (< HO, 47.7 pA, n=60; > HO, 35.0 pA, n=12), partly owing to their high input resistance (< HO, 406.7 MΩ, n=56; > HO, 452.0 MΩ, n=13). A subpopulation of SLN neurons (< HO, 8/38 cells; > HO, 1/7 cells) exhibited spontaneous spikes at rest. Activation of group I mGluRs with 3,5-DHPG (200 μM) enhanced the excitability of SLN neurons and increased their spontaneous spike rate (< HO, control, 0.71 Hz, n=37; DHPG: 4.42 Hz, n=37; paired t-test p < 0.0001; > HO, control, 0.20 Hz, n=7; DHPG: 7.24 Hz, n=7; paired t-test p = 0.0156). The enhancement was partially blocked by an antagonist for mGluR5 (MPEP, 1 μM). The modulatory effect seemed long-lasting before and shorter after hearing onset. Electrical stimulation of the area dorsal to the SLN evoked EPSCs in SLN neurons, which summated temporally to 100 Hz synaptic stimulation. Ipsilateral electrical stimulation putatively activating the input from SLN to MNTB evoked glutamatergic EPSCs in MNTB neurons. Triple immunostaining confirmed the presence of mGluR1 and mGluR5 within the SLN, revealing a heterogeneous distribution pattern surrounding calbindin1-immunoreactive neurons. Retrograde viral tracing confirmed a descending projection of SLN neurons to the ipsilateral MNTB in mice. The MNTB-projecting SLN neurons in turn receive descending projections from the inferior colliculus and auditory cortex.

Conclusions: Neurons in the mouse SLN were intrinsically highly excitable and received a glutamatergic input via a descending pathway. Neurons in the MNTB received a non-calyceal glutamatergic projection originating from the ipsilateral side. Group I mGluRs enhanced the SLN excitatory output, potentially forming a tonic excitation in MNTB neurons, which may improve their temporal processing.

SU26. Why Some Listeners Shine in the Cocktail Party: Brainstem Envelope Coding Explains It

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Background: Understanding speech in complex acoustic environments is a fundamental challenge of everyday listening. Even among individuals with clinically normal hearing, there is

wide variability in the ability to follow a target voice amidst competing talkers. This “cocktail party problem” arises from both energetic masking (overlapping sound energy) and informational masking (confusion between similar voices), yet the neural mechanisms underlying individual differences remain poorly understood. One candidate mechanism is the precision of brainstem encoding of temporal envelope periodicity, which supports the extraction of pitch cues and voice segregation. While amplitude-modulated brainstem responses reliably reflect phase-locking to periodicity, it is unknown whether individual differences in suprathreshold envelope coding predict behavioral benefits in speech-in-speech tasks versus speech in steady-state noise. We hypothesized that individual differences in suprathreshold periodicity encoding, particularly at reduced modulation depths, would correlate with behavioral measures of talker-sex release from masking, but not necessarily with performance in steady-state noise.

Methods: we measured ASSRs at 101 Hz to amplitude-modulated stimuli at 40% and 100% AM depths in a cohort of normal-hearing adults ($n=30$; mean age = 20.73 years; females=28). Neural responses were quantified using phase-locking value (PLV) obtained from Wavelet transform and signal-to-noise ratio (SNR). Behavioral performance was assessed using a two-talker speech-in-speech task, designed to elicit talker-sex release from masking, and a digit triplet in speech-shaped noise.

Results: Revealed a selective relationship: PLV at 40% AM robustly predicted the magnitude of talker-sex release from masking ($r \approx 0.35$, $p = 0.015$), whereas PLV at 100% AM and SNR measures did not. No significant associations were observed between any neural metrics and speech-in-noise performance. These findings indicate that suprathreshold envelope encoding in the brainstem captures individual differences in neural fidelity that are critical for voice segregation, but not for overcoming energetic masking.

Conclusions: Our data support the idea that precision of suprathreshold temporal encoding at the brainstem level underlies listeners’ ability to exploit pitch differences between competing talkers. The absence of a relationship with speech in speech-shaped noise performance underscores the distinct neural mechanisms for informational versus energetic masking. These findings highlight the importance of assessing suprathreshold envelope encoding when predicting speech-in-speech perception and may inform future strategies for assessment and intervention for speech-in-noise deficits in listeners with normal audiograms.

SU27. Spatial Gradient of Synaptic Input Subtypes on Bushy Cells Along the Lateral- Medial Axis of the Anteroventral Cochlear Nucleus

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Background: Bushy cells in the anteroventral cochlear nucleus (AVCN) are among the first central auditory neurons to receive input from the auditory nerve (AN). These principal cells are innervated by large, specialized synapses, known as the endbulbs of Held, from spiral ganglion neurons (SGNs) that enable precise transmission of temporal fine structure in sound. Our previous study showed that bushy neurons are innervated by various combinations of AN synapses from different subtypes of type-I SGNs. However, it remains unclear whether any spatial distribution pattern exists among different subtypes of AN synapses in AVCN bushy

neurons. In this study, we investigated whether bushy cells are spatially organized along the lateral-medial axis of the cochlear nucleus based on the distribution of different AN input subtypes.

Methods: To investigate this spatial organization, we performed immunohistochemical labeling in the cochlear nuclei of CBA/CaJ mice. Cochlear nuclei were dissected out, cryo-sectioned, and stained using antibodies against calretinin, vGluT1 and vGluT2 to probe the distribution of different AN input subtypes on bushy cells.

Results: We identified gradients of AN input subtypes along the lateral-medial axis of the AVCN. Calretinin-positive type Ia synapses were more densely expressed on the lateral side, whereas vGluT2-positive non-type Ia synapses showed higher expression medially. This spatial distribution suggests that bushy cells receive input from distinct AN subtypes depending on their position within the AVCN.

Conclusions: Our findings reveal a spatial gradient of synaptic input along the lateral- medial axis on bushy cells in the AVCN. It suggests that in addition to the tonotopic organization along the dorsal-ventral axis and the differential cell-type distribution along the rostral-caudal axis, the AVCN may also be functionally compartmentalized along the lateral-medial axis. This spatial differentiation likely contributes to the processing of complex auditory information from diverse AN inputs.

SU28. Expression of Kv4 Channels in Bushy Neurons of the Cochlear Nucleus: An Immunohistochemical Study

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Background: Bushy neurons housed in the anteroventral cochlear nucleus (AVCN) are specialized neurons that process temporal sound information critical for auditory function. These neurons possess unique biophysical membrane properties due to the expression of various ion channels. These include voltage-gated potassium channels. Our previous study used brain slice electrophysiology to isolate A-type currents mediated by Kv4 channels in most bushy neurons and revealed their role in regulating neuronal excitability and temporal precision of spikes. However, conflicting observations were reported by multiple studies, in which no A-type current was found in bushy neurons using electrophysiology. To further confirm our previous findings and characterize expression patterns of Kv4 channels in bushy neurons, we investigated the expression of Kv4.2 subunit channels in AVCN bushy neurons, using immunohistochemistry.

Methods: We investigated Kv4.2 expression in bushy cell through immunohistochemical staining and confocal microscopy in CBA/CaJ mice. Antibodies against vGluT1 and Kv4.2 subunit were utilized to identify bushy neurons and investigate their expression of Kv4 channels. Specifically, bushy neurons were identified through the innervation of end bulbs of held synapses around cell bodies labeled by vGluT1. Cell bodies were corroborated through DAPI staining. Bushy cell expression of Kv4 channels was qualitatively analyzed through Kv4.2 presence on cell bodies.

Results: Immunohistochemical staining showed that most bushy neurons of the AVCN were labeled by Kv4.2 antibody. No colocalization between vGluT1 and Kv4.2 staining was observed, suggesting that end bulb of Held synapses do not express Kv4 channels.

Conclusions: These results confirmed our previous findings that a majority of bushy neurons express Kv4 channels. These channels help shape the biophysical membrane properties and promote temporal precision of spikes in these neurons. Our study suggests that A-type currents play an important role in processing auditory information at the first neural station of the central auditory system. This is expected to impact higher auditory nuclei and promote hearing sensation.

SU29. Diffusion Tensor Imaging of Brainstem Auditory Pathway in Controls and Prospective Cochlear Implant Patients

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Background: Cochlear implants are implantable devices capable of partially restoring hearing via electrical stimulation of the auditory nerve, mimicking normal hearing conditions. Outcomes following implantation vary widely, particularly among patients with long durations of deafness. This variation limits clinicians' ability to predict who will benefit from the procedure. Neurobiological changes in the auditory pathway due to prolonged auditory deprivation may cause this variability, however, current objective markers are poorly characterized. Diffusion tensor imaging, a magnetic resonance imaging technique sensitive to white matter microstructure, offers a noninvasive means to assess subcortical auditory pathways. Some studies have reported a link between categories of auditory performance scores, but there is currently no set of biomarkers for candidate screening.

Methods: Data acquired from 23 participants were used in this project: 15 adult cochlear implantation candidates (aged 39-77) with sensorineural hearing loss, and eight high-school aged male students without reported hearing deficits as a reference group. MR imaging involved both T1-weighted and diffusion-tensor weighted scans (Patients: Philips MR7700, 1.7mm isotropic resolution; Controls: GEHC MR750, 2mm isotropic resolution).

T1 scans were registered to MNI152 space using FLIRT and FNIRT, and diffusion-weighted scans were processed using DiPy and FSL. Procedures included LPCA denoising, Gibbs ringing correction, brain extraction, and distortion correction via TOPUP and EDDY functions. White matter partial volume maps were generated via FAST and registered to DTI space. Tract masks were generated by using regions of interest (ROIs) associated with key auditory brainstem nuclei, such as the superior olivary complex (SOC), inferior colliculus (IC), and medial geniculate body (MGB) as key landmarks.

Average MNI space masks were obtained from Brainstem Navigator, registered using DiPy into both T1 and DTI space for all subjects, and averaged within population to create landmarks for

tract mapping. Probabilistic tractography was used on a subject specific basis and fit to the Constrained Spherical Deconvolution model, individually, for the left and right hemispheres. Streamlines that passed through all three ROIs were preserved.

A set of ROIs was generated from each population (Control and Patient) for the SOC, IC, and MGB. Group-specific ROIs were generated to account for variable partial volume averaging in the T1-weighted scans, ensuring preserved tractography would reside in the tissue.

Results: White matter bundle similarity was compared between the Patient and Control populations using DiPy's Bundle Analysis (BuAn) framework. No statistically significant differences were observed for these groups, but a modest linear relationship (r -squared = 0.45) was observed between the bundle similarity measure and age in the Patient population.

Conclusions: A small-sample pilot suggests a potential relationship between brainstem white matter fiber tract integrity and size and age of a prospective cochlear implantee. Further data collection is ongoing to expand the sample size and develop more uniform anatomical targets for group comparisons.

SU30. Detecting Individual Auditory Brainstem Responses (ABRs)

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Background: The Auditory Brainstem Response (ABR) is a measure of neural activity in response to sound stimuli. It is widely used for diagnosing hearing loss in humans and other animals. Current practice estimates hearing thresholds by identifying peaks in the mean ABR over hundreds of trials. However, thresholds based on the mean ABR alone decrease without bound as the number of trials used to estimate the mean increases.

We propose to score the similarity of each ABR trial with the population of ABR trials using covariance. By comparing the score distributions of stimulus-present and stimulus-absent trials, we define an ABR threshold based on the discriminability index d' . We show this measure converges as the number of trials increases.

Methods: We build a model to assess our new method, compare our new method with conventional methods, and confirm the theoretical basis for our method with a simple model and simulations.

We simulate ABR trials by generating deterministic waveforms with added Gaussian white noise. Increasing the amplitude of the deterministic waveform mimics responses to sounds of increasing stimulus level.

We then filter the simulated trials and calculate their mean for each stimulus level. At a given level, each individual trial is assigned a score equal to its covariance with the mean. The score distribution quantifies similarity between the trials.

Lastly, we compute the discriminability index d' value between the score distributions of a stimulus-present condition (non-zero waveform amplitude) with that of a stimulus-absent condition (no deterministic waveform). Because d' is the distance between the distributions, a stimulus is detected when d' is sufficiently large (for example, $d' > 1$). The stimulus level at which stimulus is detected is defined as the ABR threshold.

Results: We show that d' converges to a constant value as the number of trials increases. Consequently, ABR thresholds based on d' no longer decrease without bound as the number of trials increases. Using our simple model, we show how d' is related to the signal-to-noise ratio.

Conclusions: Previous ABR methods use the mean ABR waveform alone, causing the ABR threshold to depend on the number of stimulus trials. In this study, we introduce an alternative approach that uses covariance and d' to detect ABRs. This method defines an ABR threshold that becomes independent of the number of trials as the number increases. Future work will assess the utility of this approach using human and animal ABR data.

SU31. Enkephalin Regulation of Cochlear Nucleus Neuronal Function After Noise Exposure

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Background: While molecular and functional changes in inner ear cells post-noise exposure have received extensive attention, auditory brainstem alterations remain poorly understood. The cochlear nucleus receives signals from cochlear spiral ganglion neurons, processes/integrates them, and transmits to higher auditory centers. We show that single intense noise exposure induces pronounced molecular and functional changes in the cochlear nucleus as early as 1 day post-exposure, enriched in processes including synaptic transmission, ion channel activity, and cytoskeletal regulation. Notably, these changes persist to at least 3 days post-exposure. Concurrently, we found enkephalin plays an intriguing role here and investigated its potential cochlear nucleus protective function post-noise exposure.

Methods: We exposed 7-8-week-old C57BL/6J mice to 8-16 kHz noise at 109 dB SPL for 2 hours. The mice were then euthanized and dissected at 24 hours and 72 hours post-exposure to harvest cochlear nuclei, which were used for transcriptome sequencing or other molecular biology experiments. The results of RNA sequencing (RNA-seq) were validated using quantitative PCR (qPCR) or Western blotting (WB). Additional techniques employed included immunofluorescence staining, in situ hybridization, anterograde monosynaptic viral tracing, tissue clearing, whole-cell patch clamp recording, and auditory brainstem response (ABR) testing.

Results: Profound alterations in cochlear nucleus gene expression were observed at 1 day post-noise exposure. Enrichment analysis revealed the top enriched biological processes as synaptic reorganization, transsynaptic signaling, ion channel function, and cytoskeletal remodeling. Notably, proenkephalin (Penk) was the sole neuropeptide upregulated post-exposure, with sustained elevation through 3 days. Whole-cell patch clamp recordings localized Penk expression primarily to vertical cells of the dorsal cochlear nucleus (DCN). Via mWGA anterograde monosynaptic viral tracing, we demonstrated that Penk⁺ vertical cells innervate bushy and stellate cells in the ventral cochlear nucleus (VCN), which co-express δ - and μ -opioid receptors.

In vitro enkephalin perfusion induced hyperpolarization of resting membrane potential and reduced action potential firing in VCN neurons, suggesting a potential protective role against noise-induced hyperexcitability. Pretreatment with the δ -opioid receptor agonist SNC80 prior to noise exposure significantly ameliorated ABR threshold elevations, along with improved wave II latency and amplitude at 3 days post-exposure. Transcriptome sequencing and WB validation further revealed that δ -opioid receptor activation exerts neuroprotective effects—including anti-inflammatory and anti-apoptotic actions—via pathways including PI3K-AKT, MAPK, and NF- κ B.

Conclusions: A single intense noise exposure induces profound changes in gene expression in the mouse cochlear nucleus and upregulates proenkephalin (Penk) expression in vertical cells of the DCN. This upregulated Penk, via axonal innervation, modulates the electrophysiological properties of neurons in the VCN that express enkephalin receptors, including δ - and μ -opioid receptors. Prior to noise exposure, intraperitoneal administration of the δ -opioid receptor agonist SNC80 exerts neuroprotective effects in the cochlear nucleus—including anti-inflammatory and anti-apoptotic actions—and improves ABR metrics at 3 days post-exposure.

SU32. Triple AAV Vectors Enable Efficient and Safe Cochlear Gene Delivery for Large Genes in Adult Mice

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Background: Several reports have demonstrated auditory restoration after intra-cochlear gene therapy in patients with OTOF-related hearing loss. However, as nearly 200 causative genes have been identified in hereditary deafness, expanding the scope of target genes for cochlear gene therapy is necessary. Most studies of hereditary hearing loss employ adeno-associated virus (AAV) vectors, but their carrying capacity is limited to ~4.7 kb. Because some deafness genes exceed this size, packaging into a single AAV capsid is unfeasible, and splitting the transgene into two or three parts may be required. While successful dual-AAV-vector approaches have been reported, no study has yet demonstrated the feasibility of triple-AAV delivery. We therefore aimed to establish a triple-AAV cochlear delivery strategy that achieves apex-to-base transduction with high efficiency and minimal cochlear damage.

Methods: Enhanced green fluorescent protein (EGFP) was divided into three parts and packaged separately into AAV2/2 vectors (each titer > 10¹³ GC/ml; VectorBuilder, construct patent pending). C3H/HeNjcl mice (5–6 weeks old) were used. In the left ear, 1 μ l of the triple AAV mixture was co-injected into the cochlea using the round window membrane + canal fenestration (RWM+CF) method we previously described. Auditory thresholds were assessed by auditory brainstem response two weeks post-injection. Cochleae and vestibular organs were then harvested and dissected to evaluate transduction efficiency.

Results: Robust transduction (40–50%) was observed in inner hair cells (IHCs) across all cochlear turns (apex, middle, and base). In contrast, outer hair cells (OHCs) and vestibular hair cells (utricle, saccule, semicircular canals) were not transduced. Importantly, auditory thresholds did not differ between injected and uninjected ears, indicating that the procedure did not affect hearing.

Conclusions: This study is the first to demonstrate that cochlear gene delivery using triple-AAV vectors is both efficient and safe for transducing IHCs in the adult murine cochlea. These findings suggest that triple-AAV strategies could be broadly applicable to the development of gene therapies for hereditary hearing loss caused by large deafness genes, such as CDH23, MYO15A, and USH2A that exceed the packaging limit of a single or dual AAV vector.

SU33. GJB2 Gene Therapy-Response of Two Pre-Clinical Mouse Models of the Most Frequent Form of Human Deafness, DFNB1

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Background: More than 1.5 billion people worldwide live with hearing loss (HL). Sensorineural HL affects one new-born in 700-1000 and approximately one child or young adult in 500 before the age of 20. Approximately 70% of congenital severe to profound deafness cases are hereditary due to monogenic defects, thus potentially treatable by gene therapy (GT).

GJB2-related autosomal recessive non syndromic HL (also referred as DFNB1) is the most common genetic cause of congenital sensorineural HL in many human populations, frequently accounting up to half cases. In addition, it is also responsible for late onset deafness, occurring even after 40 years of age. GJB2 encodes gap-junction channel protein connexin-26 (Cx26), involved in metabolites and ions homeostasis in the cochlea, necessary for cochlear development and sensory hair cell function and survival. Notably, Cx26 contributes to the endocochlear potential, the driving force of the sensory mechanotransduction. Hundreds of pathogenic GJB2 variants including large deletions, loss-of-function, and missense variants have been reported which outcomes spanning from mild to profound deafness.

Methods: To develop GT approaches for deafness caused by Gjb2 defects, we generate two conditional knockouts mouse models (as Gjb2 inactivation results in embryonic mouse lethality) crossing transgenic Gjb2loxP mice with Cre mice which display strong and wide Cre recombinase cochlear expression (i.e. in almost all Gjb2-positive cells). Both models display profound congenital deafness, severe cochlear architecture abnormalities and reduced endocochlear potential

Results: ear delivery of a GT-Gjb2 recombinant adeno-associated virus (AAV) results in a strong and long lasting (at least 6 months) Cx26 expression in most of Cx26 expressing cells of the cochlea especially in Hensen's cells, Deiters' cells, inner and outer pillar cells, inner phalangeal cells, inner border cells and inner sulcus. Importantly, no Cx26 was detected in hair cells in any of the 2 models. In both models, GT-Gjb2 improves ABR threshold up to 3 months post administration and preserve cochlear structure in almost 90% of mice. Furthermore, GT-GJB2 strongly reduce hair cells degeneration and neurons loss.

Conclusions: Thus, GT-GJB2 improves audition in these preclinical models of Gjb2 defects

SU34. High-Throughput Screening Uncovers the Hair Cell-Specific Promoter for Gene Therapy

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Background: Hearing loss is the most common sensory disorder globally, affecting over 400 million people, including 34 million children. Approximately 1 in 500 newborns suffer from hearing impairment, with genetic factors accounting for about 60% of cases. Adeno-associated virus (AAV)-mediated gene therapy has emerged as a promising treatment strategy for genetic hearing loss. However, the complexity of the cochlea—particularly its diverse cell types such as hair cells, supporting cells, and spiral ganglion neurons—poses significant challenges to precise gene delivery targeting. This study focuses on the systematic screening and identification of specific promoters for inner ear hair cells. By combining AAV vectors with high-throughput promoter screening, we achieved targeted delivery and precise expression in different types of cochlear hair cells, effectively overcoming previous issues such as off-target effects, low safety, and high dosage requirements in viral delivery. This work provides critical technical support for the clinical translation of genetic therapies for hereditary hearing loss.

Methods: Utilizing cochlear single-cell sequencing data, we conducted bioinformatic analysis and molecular cloning to construct a diverse hair cell promoter library encompassing various subtypes. A two-phase screening strategy was employed: high-throughput primary screening with a fluorescent reporter system identified candidate promoters, followed by precise secondary evaluation of their strength and specificity. An AAV-based gene therapy system was microinjected into *Pjvk*^{-/-} mice inner ears. Auditory function was assessed using ABR and DPOAE measurements, while immunohistochemistry evaluated protein expression and cell survival to determine system efficacy and safety.

Results: Through systematic screening, specific promoters for different types of hair cells were identified, enabling highly efficient and specific EGFP expression in hair cells. Based on one of these hair cell-specific promoters, HCpro, an AAV-mediated gene therapy system targeting hair cell-related deafness gene was designed. This system was delivered via microinjection into the inner ear of *Pjvk*^{-/-} mice. Auditory function was evaluated at multiple points post-treatment, revealing significantly reduced response thresholds compared to the untreated control group. Furthermore, no obvious adverse effects were observed in the cochleae of the treated mice, demonstrating the favorable safety and efficacy of this AAV-based gene therapy system. Ultimately, the treatment successfully restored auditory function in this genetic model of hearing loss.

Conclusions: Distinct from conventional promoter screening approaches, this study established an efficient and systematic strategy for identifying hair cell-specific promoters and successfully identified HCpro. This work not only provides a novel promoter option for the design of gene therapies targeting hair cell-related deafness genes but also opens avenues for extending

treatment strategies to other cochlear cell types, such as supporting cells and spiral ganglion neurons.

SU35. A Directed Evolution Novel Aav Mediated Gene Therapy Restores Hearing in Otof Mutant Mice and Patients

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Background: OTOF commonly causes non-syndromic auditory neuropathy, that affects 2.3-10% of patients with hereditary hearing impairment. OTOF only expresses in inner hair cells (IHC), crucial in synaptic vesicle cycling. Dual AAV mediated gene therapy has successfully restored hearing in patients with OTOF mutations. AAV is a single-stranded linear DNA defective virus with multiple serotypes targeting different tissues or cells. IHC-specific AAV vector still remains a major challenge.

Methods: In this study, we developed a platform to engineer novel AAV vectors through capsid mutation, peptide insertion, DNA shuffling, and promoter modification. A novel AAV vector, AAV-WM04, was engineered, which exhibits extremely high specificity for IHC in both mice and non-human primates (NHPs). Based on this vector, a dual-vector OTOF expression system was designated as EA0010.

Results: EA0010 was injected into inner ear through round window in adult wild type mice and NHPs for safety verification. EA0010 rescued hearing of humanized adult OTOFQ829X/Q829X mice. To evaluate the effectiveness of EA0010 in patients with severe-to-profound hearing loss caused by OTOF gene mutations, we designed a single-center, single-arm, open-label, dose-escalation study. From Dec 2024, nine patients have been enrolled in this study.

Conclusions: Preliminary clinical results demonstrate that EA0010 effectively restores auditory function, especially in restoring high-frequency hearing, with no serious adverse event. Detailed results will be presented.

SU36. A Novel AAV Vector-Based Gene Therapy for Two Adult Rodent Models of DFNB1

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Background: GJB2-related autosomal recessive nonsyndromic HL (DFNB1) is the most common genetic cause of congenital sensorineural HL worldwide, accounting for up to half of all cases in some populations. It can also cause later-onset HL, even after 40 years of age. GJB2 encodes connexin-26 (Cx26), a gap-junction channel protein critical for metabolite and ion homeostasis, cochlear development, and sensory hair cell function and survival. Hundreds of pathogenic GJB2 variants have been identified, ranging from large deletions to missense and loss-of-function mutations, with clinical outcomes spanning mild to profound deafness.

Methods: We developed AAV-DS1, an AAV vector optimized for DFNB1, designed for broad coverage of Gjb2-expressing cells in the inner ear of mouse, rat and minipig, while minimizing transduction of hair cells. AAV-DS1 with GJB2-specific promoter to drive the expression of wild-type GJB2 (AAV-DS1-GJB2) was delivered into the inner ears of adult Gjb2-mutant mice and rats via round window (RW) injection. Ongoing studies investigated efficacy, dose-response, correlation between ABR responses and transduction efficiency, early biodistribution, and toxicology in mice. In parallel, AAV-DS1-GJB2 was administered to minipigs using a clinically relevant surgical approach and medical device. Tolerability and biodistribution were assessed, and AAV tropism was characterized by immunoconfocal analysis and in situ hybridization on cochlear whole mounts and cryosections.

Results: A single intracochlear injection of AAV-DS1-GJB2 in adult Gjb2-mutant rats and mice resulted in a significant, dose-dependent improvement in auditory thresholds as early as 2 weeks post-injection. In minipigs, ABR thresholds and DPOAE amplitudes remained within the normal range 6 months post-surgery, confirming local tolerability. Biodistribution was largely restricted to injected ears. In WT mice, AAV-DS1-GJB2 was well tolerated, with no ABR threshold shifts up to 6 months and evidence of long-term transgene expression. Across rodents and minipigs, anti-HA labeling confirmed broad targeting of nearly all GJB2-expressing cell types without adverse functional impact.

Conclusions: AAV-DS1-GJB2 efficiently targets endogenous GJB2-expressing cochlear cells, with demonstrated efficacy and favorable safety in rodents and minipigs. These findings provide strong support for advancing AAV-DS1-GJB2 into clinical translation—enabling studies as a novel gene therapy for GJB2-related hearing loss.

SU37. Triple Antisense Oligonucleotide-Mediated Exon Skipping Targeting a Large Deletion in USH2A

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Background: Usher syndrome is an inherited autosomal recessive condition that accounts for about 50 percent of all hereditary deaf-blindness cases. There are three types of Usher syndrome, of which Type 2 is the most common. USH2 is characterized by moderate to severe sensorineural hearing loss, nyctalopia, and progressive loss of vision. The most common form, USH2A is caused by mutations in the USH2A, a 15.7 kb gene, that encodes for the protein usherin, expressed in cochlear hair cells and photoreceptor cells.

A patient at Boston Children's Hospital was diagnosed with USH2A. Genetic testing revealed two pathogenic mutations: an in-frame deletion of exons 61-63 that results in improper protein folding and a single base-pair deletion leading to the production of a truncated protein. Using Alpha Fold, we structurally analyzed the protein harboring the three-exons deletion and determined that skipping an additional three exons upstream of the deletion may restore proper protein folding and function. As such, we propose to develop antisense oligonucleotides (ASOs) that target these three exons and lead to removal of the region encoded by six consecutive exons, exons 58 to 63. This shortened protein excludes part of fibronectin (FN) domain FN22, all of FN23 through FN30, and part of FN31. The objective of this project is to determine whether the larger USH2A truncation restores function.

Methods: Exon skipping is a method in which ASOs are used to prevent the interaction between mRNA and the ribosome to elude translation. We designed several ASOs targeting splice sites in USH2A exons 58, 59, and 60, and assessed their skipping in retinoblastoma cells at a concentration of 200 nM for 48 hours. Skipping was evaluated by RT-PCR and sequencing. Patient-derived induced pluripotent stem cells (iPSCs) have also been generated using blood sample from the proband. USH2A expression and splicing in the USH2A iPSCs line is being assessed via RT-PCR.

Results: Primers were designed and validated in RB1 cells. RB1 cells were transfected with ASOs at 200 nM for 48 hours and subsequently harvested. RT-PCR was performed with the different primer sets. We validated skipping of exons 58 and 59 and are currently assessing other ASOs targeting exon 60. We are also assessing multiple exon skips combining different ASOs. Additionally, we are assessing mutant iPSC lines by RT-PCR to confirm splicing in absence of ASOs. We are also developing iPSCs derived inner ear and retinal organoids to test validated ASOs in the context of the three exons deletion.

Conclusions: This strategy is novel in that we are assessing skipping of 3 exons in the presence of another 3-exon deletion. We predict that removal of a large portion of the protein encoded by USH2A will lead to expression of a shorter, yet functional protein.

SU38. AAV Delivery of Cib2 Restores Hearing and Vestibular Function in Cib2 Ko Mice

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Background: Calcium and integrin-binding protein 2 (CIB2) binds to TMC1, the pore forming complex of the mechanotransduction channel (MET) and is essential for hearing. Mice lacking both Cib2 and Cib3 double knockout mice also show impaired vestibulo-ocular reflexes, head bobbing, and poor balance. Cib2 and Cib3 single knockout mice appear phenotypically normal; however, some reports of subtle progressive balance phenotypes have been reported in single KO mice by 9 months of age, along with stereocilia disorganization that may be associated with progressive degeneration. Patch clamp recording from vestibular hair cells suggest that Cib2 and Cib3 are functionally redundant in regulating MET. However, RNA expression of Cib2 is predominantly in striolar hair cells whereas Cib3 in extrastriolar hair cells.

Methods: Here we use vestibular evoked myogenic potentials (VsEP), rotarod, open field, and infrared palm reader analysis of Cib2 and Cib3 single KO mice to assess balance phenotypes in mice. We also used auditory brainstem response (ABR) to examine restoration of auditory function in injected mice. Hairpin chain reaction fluorescent in situ hybridization was used to assess Cib2 and Cib3 expression relative to the Ocm⁺ striolar domain and quantify in cellular resolution. RT-qPCR was completed to assess the relative distribution of Cib2 and Cib3 separately in utricles, saccules, crista, and cochlea to compare to HCR quantitative analysis. Functional assessment of mechanotransduction in Cib2 KO and Cib3 KO tissue was completed using FM1-43 labeling of acutely dissected tissues.

Results: Here we show that Cib2 KO and Cib2/3 DKO mice fail to produce VsEP responses to head jerk stimuli, whereas Cib3 KO mice have normal VsEP responses under the same conditions. Unilateral neonatal utricle injection of AAV is sufficient to restore Cib2 KO VsEP

and ABR responses to near WT thresholds in both ears. Similar to other groups, we identify no significant behavioral balance phenotype in Cib2 or Cib3 single KO mice. HCR-FISH shows non-redundant expression patterns of Cib2 in the striola and Cib3 in the extrastriola in the utricle and saccule. Similarly, Cib2 KO tissue shows FM1-43 dye absent in the striola of the vestibular maculae.

Conclusions: Taken together, these data indicate that Cib2 expression in the striola is necessary for VsEP generation. Additionally, despite its expression in vestibular organs, Cib3 it cannot compensate for loss of Cib2 when it comes to VsEP generation or striolar MET function. These results suggest that Cib2 and Cib3 are not functionally redundant in the vestibular system. Despite the regional differences in their expression, our behavioral phenotyping does not indicate a definitively distinct role for Cib2 versus Cib3 in balance. Thus, the underlying reasons for the non-overlapping expression and function of Cib2 and Cib3 remain an interesting area for future study.

SU39. Investigation of Vestibular Dysfunction Requires Renewed Research Enthusiasm

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Background: Hearing, balance, ocular gaze, head position, baroreflexes, and spatial memory all depend on functional input from structures of the inner ear to the brain. Close examination of scientific research output based on anatomical search terms of peripheral inner ear structures shows an obvious lag in areas of vestibular research progress in areas of academic publication, grant funding, and clinical trial development.

Methods: We utilized data mining of publicly available records (PubMed, NIHReporter, and ClinicalTrials.gov) to assess the rate of inner ear research output quantitatively. PubMed and NIHReporter searches included keyword terms separated by Boolean operators to focus on either auditory or vestibular anatomical peripheral structures with suffixes excluded for more inclusive output metrics. Similarly, we queried clinicaltrials.gov with additional search terms included pertaining to symptoms of inner ear dysfunction and names of syndromes. Query results were categorized into either vestibular (e.g. dizziness, vertigo, imbalance, etc.) or auditory (e.g. hearing, deafness, presbycusis, etc.). We combined these computational approaches with systematic review of published literature including patient survey data to understand the prevalence of hearing loss versus dizziness. We also reviewed reported cost estimates from multiple studies regarding unexplained falls to understand the relative cost of untreated vestibular dysfunction.

Results: PubMed data show that the expansive growth period of inner ear research by publication output from 1950 to 2024, particularly in cochlear research. In contrast, fewer publications contain peripheral vestibular anatomical terms ranked by frequency: saccule > utricle > otolith > semicircular canal > vestibular labyrinth > crista ampullaris > vestibular macula. Publications containing auditory terms ranked by frequency: cochlea > organ of corti > spiral ganglia > basilar membrane > stria vascularis > tectorial membrane > Reissner's membrane. NIHReporter queries from 1990-2024 reveal thousands less grants are awarded to projects focused on vestibular research compared to auditory/cochlear research. Unsurprisingly,

the total dollars spent on auditory/cochlear research surpasses vestibular research investment by a significant margin. Interestingly, our review of various patient survey studies show prevalence estimates between dizziness and hearing loss to be comparable.

Conclusions: Vestibular research consistently receives less public attention in the form of published research output, grant funding, and clinical trial development. While there are unique challenges associated with diagnosing patients with vestibular dysfunction, our prevalence estimates of dizziness appear comparable to hearing loss. During literature review, it became clear that vestibular dysfunction had far worse impacts on patients' quality of life and may have higher diagnostic and treatment costs than hearing loss as well. Despite a substantial need for clinical diagnostic and therapeutic interventions for vestibular dysfunction, research in this area is severely limited. A renewed enthusiasm among the next generation of trainees to investigate the vestibular system may help facilitate broader understanding of the inner ear and produce interventions for patients.

SU40. Catalyzing Hearing Research With the Gene Expression Analysis Resource (umgEAR.org)

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Background: The gene Expression Analysis Resource (gEAR, umgear.org) is an online portal for multi-omic data visualization, sharing, and discovery, and continues to be an invaluable public resource for the hearing research community. gEAR allows for visualization, investigation, and reuse of sequencing datasets in a user-friendly web interface. The availability of hearing datasets in gEAR continues to grow with 1,299 datasets, 2,557 unique users, and over 3.75 trillion individual datapoints.

Methods: The proliferation of single cell/nuclei, epigenomics, multiome, and spatial transcriptomics techniques in the hearing research field has led us to incorporate additional visualization and analysis tools which provide users the ability to explore and analyze these datasets using only a web browser with no coding required. Expanded epigenomic tools allow users to quickly visualize open chromatin regions and methylation data alongside gene expression and other dataset displays.

Results: In addition to the range of visualization and search functionality offered via gEAR's new Epigenetic Explorer tools, epigenetic datasets in gEAR now dynamically link to the popular UCSC genome browser for even more analysis options. New spatial tools allow for the upload and interactive exploration of data from all major commercially available spatial platforms (e.g. 10X Visium and Xenium, CosMx and Curio, and more). Similar to other tools in gEAR, the spatial layouts now can be viewed within a dataset collection allowing for correlated views with other popular datasets.

Conclusions: In contrast with other online data visualization tools, a major strength of the gEAR platform is the ability to visualize, curate, and explore multiple datasets and data types side-by-

side. New options for publication landing pages (new pages with custom content to highlight your manuscript and link to all your data), dataset collection customization, and easily implementable transfer learning tools help researchers dramatically increase the visibility, interpretability, and reuse of their sequencing datasets and explore them alongside other cutting-edge hearing datasets. For users visiting gEAR for the first time, gEAR now includes interactive walkthroughs introducing users to the major functionalities of the platform and use of individual tools. gEAR is supported by the National Institute on Deafness and Other Communication Disorders (NIDCD), Hearing Health Foundation (HHF), and the Hearing Restoration Project (HRP) to promote discovery in hearing research.

SU41. Data-Driven Subtyping of Tinnitus Patients Using Deep Embedded Clustering

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Background: Tinnitus is a heterogeneous auditory disorder characterized by diverse clinical manifestations and variable therapeutic responses. While Tinnitus Retraining Therapy (TRT) has been widely implemented, its outcomes differ substantially among patients, reflecting the multifactorial nature of tinnitus involving auditory, psychological, and neurobiological dimensions. Conventional classification systems often fail to capture this complexity, limiting the precision of diagnosis and the prediction of treatment outcomes. Deep learning, particularly deep embedded clustering, provides an opportunity to identify latent structures within multidimensional clinical data and uncover novel subtypes of tinnitus that may guide personalized therapy. The present study aimed to utilize a deep embedded clustering approach to stratify tinnitus patients undergoing TRT into data-driven subtypes, characterize their clinical and audiometric profiles, and examine differential therapy outcomes across subgroups.

Methods: A retrospective dataset of patients treated with TRT was analyzed, including tinnitus handicap inventory (THI) scores, visual analogue scale (VAS) ratings of loudness and annoyance, audiometric thresholds across frequencies, tinnitus psychoacoustic features, psychological stress (BEPSI) and depression (BDI) indices, and therapeutic histories. Representation learning was performed using an autoencoder to reduce high-dimensional inputs into latent features, which were subsequently clustered using k-means in the embedded space. Subgroup characteristics were examined using descriptive statistics and visualized in latent manifolds. Longitudinal treatment responses, assessed through serial THI and VAS scores, were modeled to evaluate the clinical validity of the identified clusters.

Results: Deep embedded clustering revealed three clinically distinct patient subtypes. The first cluster was defined by frequency-specific hearing loss with moderate tinnitus severity, the second by high psychosocial burden with elevated stress and depression indices, and the third by predominantly tonal tinnitus with relatively preserved hearing. These subgroups demonstrated differential TRT trajectories: patients in the psychosocially burdened cluster exhibited slower recovery and higher residual symptoms, whereas those with tonal tinnitus and minimal hearing impairment showed more rapid and sustained improvement. Feature importance analyses further indicated that baseline THI, stress levels, and high-frequency hearing thresholds were the strongest predictors of subgroup membership and therapeutic outcomes.

Conclusions: This study demonstrates that deep embedded clustering can uncover hidden subtypes of tinnitus patients beyond conventional diagnostic categories. By integrating heterogeneous clinical, audiometric, and psychological features, this data-driven approach provides mechanistic insights into tinnitus heterogeneity and offers a framework for personalized management. Importantly, the identification of subgroups with distinct TRT outcomes underscores the clinical utility of artificial intelligence in guiding patient-specific therapy. These findings highlight the potential of AI-driven diagnostic and therapeutic platforms to bridge complex clinical data with translational neuroscience, advancing the precision of tinnitus care and contributing to a deeper understanding of its underlying pathophysiology.

SU42. Minipig Model Development for Hearing Research

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Background: The inner ear drug, biologics, and device development field has been limited by the lack of large-animal models to effectively bridge the gap between rodents and humans. Here we propose the miniature pig (Sinclair NanoPig) as an alternative large animal model in translational hearing research. Compared to commonly used large animal models such as dogs, cats, and some NHPs, the minipig cochlea dimensions are more similar to humans, enabling preclinical studies that use human-relevant dose volumes for studying drug distribution in the cochlea as well as human-sized implantable devices. Further, use of minipigs as a large animal model in biomedical research is cost-effective and in accordance with recent regulatory guidance to limit the use of NHPs in preclinical research. The minipig holds great promise as a better translational model for hearing research, but in order to expand their use in preclinical hearing research studies, there is a need for refining surgical access for delivery and sampling as well as development of normative audiometric datasets and behavioral audiometry methods that mirror clinical outcome measures of sound perception and audibility.

Methods: We used minipigs to develop an operant sound detection task utilizing a custom touchscreen device. Animals were trained to press illuminated boxes on a touchscreen in response to sound or silent stimuli in a two-alternative forced choice task. In addition to behaviorally assessed hearing thresholds, we have generated normative datasets for both bone- and air-conduction ABRs. Preliminary pharmacokinetic studies attempted perilymph sampling from the cochlear apex but found the approach technically difficult. Simulations showed that sequential perilymph sampling from the lateral semi-circular canal, as used extensively in guinea pigs, provides sufficient detail to document drug distribution.

Results: Through this method development study, we have demonstrated the feasibility of a touchscreen sound detection task in the minipigs and have refined local surgical access surgical approaches for drug delivery and sampling. We have also optimized key parameters for DPOAE and ABR measurement in mini pigs.

Conclusions: The minipig is an alternative large animal model that can effectively bridge the translational gap between rodents and humans due to the similarity in cochlear dimensions and anatomy to the humans. Optimizing perilymph sampling procedures and ABR and DPOAE measures in the minipig and developing behavioral assays that closely approximate outcome

measures in humans such as the audiogram or speech-in-noise tests, further aids in the translational validity of the minipig in auditory preclinical research.

SU43. Universally Controversial Stimuli Reveal That Adversarial Robustness Improves DNN Prediction Accuracy Across the Entire Human Auditory Cortex

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Background: Sensory neuroscientists seek to develop models that replicate the computations of sensory systems and predict responses to complex stimuli. A key challenge is that distinct computational models often make similar predictions for complex natural stimuli, such as speech, masking potentially important computational differences. Prior research found that artificial deep neural networks (DNNs) trained on challenging auditory tasks learn features that predict much of the human cortical response to natural sounds. However, the decisions of DNNs can diverge sharply from human behavioral decisions. For example, standard DNNs make confident categorization judgments for noise-like sounds that are completely unrecognizable to a human observer. Training DNNs to be robust to adversarial attacks better aligns model decisions with human judgments. Despite this behavioral difference, standard and robust DNNs make nearly identical predictions for human cortical responses to natural sounds. Here, we develop a new approach for model comparison to investigate these seemingly contradictory results.

Methods: We developed a method to synthesize sounds that generate universally controversial predictions across the entire human auditory cortex and leveraged this method to compare standard and adversarially robust DNNs. Using fMRI data from 30 participants listening to 165 natural sounds (2 seconds each), we fit voxel-wise encoding models by linearly mapping DNN layer activations to fMRI voxel responses, separately for a standard and robust DNNs trained to recognize spoken words. We then developed a synthesis algorithm that generated a single set of 60 sounds that produced uncorrelated predictions across all auditory voxels. Our synthesis procedure successfully generated perceptually diverse, complex sounds—some speech-like, others tonal or noise-like. We then measured fMRI responses to these controversial sounds along with 120 natural sounds from 9 new participants.

Results: We found that standard and robust DNNs predicted fMRI responses to natural sounds with nearly identical accuracy. Moreover, the predictions from standard and robust models were themselves very similar for natural sounds, virtually guaranteeing similar neural prediction accuracy. In contrast, we found that controversial sounds produced uncorrelated model predictions throughout the entire human auditory cortex, even in participants whose data was not used to synthesize the sounds. We found that the robust model maintained high prediction accuracy for measured fMRI responses to the universally controversial sounds, while the standard model's accuracy dropped to near zero. We also found that natural and controversial sounds share a low-dimensional neural manifold along which standard and robust DNNs make highly controversial predictions.

Conclusions: We provide a general-purpose method to synthesize stimuli that distinguish competing sensory models. It is applicable to virtually any model, modality, or recording

technique. Our results demonstrate that adversarial robustness substantially improves neural prediction accuracy and enables more effective population control along the neural manifold.

SU44. A Multi-Channel Integrated Auditory Function Test System

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Background: ABR is the gold standard for objective hearing assessment of animals and people. The extensive use of genetically engineered mice in hearing research has created an urgent demand for the ability of ABR high-throughput testing.

Methods: We developed a four-channel ABR system enabling simultaneous testing of four subjects, integrating distortion product otoacoustic emission (DPOAE), cochlear microphonic (CM) and endocochlear potential (EP) detection functions.

Results: The system adopts high-speed ADC chip, which can obtain DPOAE and CM data above 32kHz. For the detection of various peak parameters (amplitude and latency) of mouse ABR, the performance is equivalent to that of the industry standard equipment TDT, and the test efficiency is improved by three times

Conclusions: This multifunctional test platform meets the current high-throughput ABR testing requirements, and provides excellent efficiency and expanded functional evaluation for the scalable high fidelity auditory phenotype in the next generation research.

SU45. Irregular Afferent Pathways Associated With the Striola Are the Peripheral Origin of Motion Sickness

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Background: At present, the pathological mechanism of motion sickness (MS) focuses on the mismatch between the real sensory input and the self-motion estimation predicted by the "internal mode". Drugs for the treatment of MS have obvious side effects due to a wide range of central action targets. Basic medical evidence shows that the pathological origin of MS can be traced to the abnormal signal input of vestibular receptors. Can we establish an interpretation model of the mechanism of MS through the analysis of the regulation path of peripheral vestibular input?

Methods: In this project, we studied the vestibulo-ocular reflex (VOR) of CIB2^{-/-} and CIB3^{-/-} transgenic mice expressed in the striola and extra-striola zone of otoliths, including VOR-SPN (Slow phase nystagmus) and VOR-FPN (Fast phase nystagmus). After that, MS in mice was induced by biaxial rotation, and the severity of MS was evaluated using real-time body temperature monitoring (core body temperature and tail temperature), VOR decline after rotation, and swimming posture control after rotation.

Results: (1) VOR-SPN mainly reflect the function of extra-striatal zone, which shows that the slow phase eye movements of aVOR (angular VOR) and OVAR (Off-vertical axis rotation) in

CIB3^{-/-} mice are significantly reduced. CIB2^{-/-} mice have no obvious vestibular dysfunction symptoms, including VOR and VSR.

(2) Compared with the control group C57 BL/6J mice, CIB2^{-/-} mice don't show significant thermoregulatory response during rotational stimulation, and their VOR efficacy was higher than that of the control group after rotation, with normal swimming posture.

Conclusions: The irregular afferent pathway associated with hair cells in the striola zone is the main vestibular peripheral source of MS. This discovery precisely locates the origin of MS from the macroscopic vestibular system to the sub organ structure - the striola zone. Thus, we will shift the targeted diagnosis and treatment of MS from the central system to regulating specific peripheral signaling pathways.

SU46. Resurgent Sodium Currents and Nav1.6 Expression in Vestibular Calyx Afferent Terminals

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Background: Vestibular afferent neurons relay signals from hair cells to the brain. Calyx-only afferents innervate type I cells, bouton afferents target type II cells, and dimorphic afferents contact both hair cell types. Firing characteristics vary by zone, with dimorphic afferents in the peripheral zone (PZ) showing highly regular repetitive activity. Ionic conductances, including sodium currents—transient (INaT), persistent (INaP), and resurgent (INaR)—may shape firing properties. INaR, generated by an open-channel block mechanism supports repetitive firing in select neurons, but its role in vestibular afferent signaling is unclear. We therefore used whole-cell patch-clamp to investigate INaR in vestibular calyces and modeling and immunohistochemical approaches to investigate underlying channels.

Methods: Cristae were extracted from Mongolian gerbils (P30-42), embedded in agarose, and sliced at 100 μ m. The central zone (CZ) and PZ regions were identified, and calyx terminals distinguished by their cup-shaped morphology. Whole-cell patch-clamp was used with K⁺- or Cs⁺-based intracellular solutions. A series of voltage protocols was applied to characterize INaT and INaR in voltage clamp. Action potentials were recorded in current clamp using K⁺-based electrode solutions. Na⁺ currents were modeled with a Markov kinetic scheme (Hong et al. 2018). For immunohistochemistry, slices were fixed and stained with anti-CASPR1 and anti-Nav1.6 antibodies and fluorescent images viewed on a confocal microscope.

Results: In whole-cell recordings all calyces expressed INaT. INaR was present in a subset of calyces, with 19 out of 38 PZ calyces (50%) and 4 out of 16 CZ calyces (25%) exhibiting INaR. Both current components were blocked by tetrodotoxin (500 nM). Preliminary data demonstrate that INaT in calyces expressing INaR activate at more negative potentials compared to those lacking INaR suggesting different underlying subunits. INaR amplitude depended on the duration and voltage of the preceding depolarizing step: brief (10 ms) or larger (+30 mV) depolarizing prepulses enhanced INaR compared to longer (50 ms) or smaller (−10 mV) steps, consistent with open-channel block and slow inactivation dynamics. INaR⁺ calyces exhibited more depolarized half-inactivation potentials for the INaT ($V_{1/2} = -72.4$ mV) than INaR[−] calyces

($V_{1/2} = -85.7$ mV). INaR facilitated faster recovery from both open-channel block and inactivation, and our computational model replicated voltage clamp findings. In current clamp, calyces with INaR displayed multiple spikes whereas those without INaR typically showed single evoked spikes. Anti-CASPR1 stained calyces and heminodes adjacent to anti-Nav1.6 staining below the neuroepithelium.

Conclusions: We found that INaR is prevalent in PZ calyces of the crista. Calyces with INaR exhibit faster recovery from inactivation and open-channel block, more depolarized half-inactivation of INaT, and repetitive firing. Nav1.6 channels may contribute to the generation of INaR within dimorphic calyx afferents. Understanding the heterogeneity of Na⁺ channels and firing mechanisms in vestibular afferents could guide treatment for vestibular dysfunction.

SU47. Effect of Modulus Presentation on the Magnitude Estimates of Angular Velocity

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Background: Magnitude estimation is a psychophysical method in which participants assign numerical values to physical stimuli. This method can be used to measure the perceived intensity of vestibular sensory experiences, such as angular motion. A reference stimulus, or modulus, is commonly used as an initial reference to which a specific number is assigned. Subsequent stimuli are then assigned numbers that reflect the participant's perceived magnitude relative to the modulus. While use of a modulus during magnitude estimation of various sensory experiences was used in the original method by S.S. Stevens in the 1950's, not all applications of magnitude estimation use a modulus in the same way. To date, the effect of modulus presentation on magnitude estimation of vestibular stimulation is not understood. The purpose of this study was to examine whether the scaling of a sinusoidal harmonic acceleration stimulus presented at varying peak velocities changed with the presentation of the modulus.

Methods: Twenty-one healthy adults (mean age = 20.5 years) estimated the magnitude of a single cycle sinusoidal harmonic acceleration stimulus presented at .16 Hz in 9 randomly presented peak velocity conditions (10-90 dps). A modulus was presented to all participants and assigned a value of 50. Participants were randomly assigned to one of three modulus groups: (1) modulus presented once at the beginning, (2) modulus presented at the beginning and again at midpoint, or (3) modulus presented before each condition. The dependent variable was the slope of the psychophysical function.

Results: Across groups, perceived angular velocity followed a log-linear function consistent with Stevens' Power Law. The exponents of perceived angular velocity varied between .72 and .81. Statistical analyses revealed no significant group differences.

Conclusions: The psychophysical function obtained by the method of magnitude estimation was not significantly influenced by the modulus presentation as a compressive scaling function was observed regardless of modulus presentation frequency. The results support the use of a single modulus, presented at the beginning. Future work should examine conditions without a modulus (i.e. "free modulus" where participants choose their own reference numbers) and whether there are interactions between the magnitude of the modulus and the psychophysical function. Future work should also examine if these results hold true in different participant cohorts, particularly

older participants or those with vestibular loss. Key take-away: The frequency in which a modulus was presented did not significantly affect the psychophysical function obtained by the method of magnitude estimation of angular motion.

SU48. Fall Risk in Underserved Populations

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Background: Falls are a leading cause of morbidity among older adults, yet disparities in fall risk remain poorly understood in underserved populations. Standardized tools such as the CDC STEADI (Stopping Elderly Accidents, Deaths, and Injuries) provide a validated framework for community-based screening but do not incorporate audiologic or vestibular risk factors. In the Harrisonburg, VA community, approximately 20% of residents identify as Hispanic, a proportion nearly double the statewide average, making the inclusion of this population especially important for fall risk screening. The proposed study will enhance the STEADI model by integrating vestibular and hearing assessments along with social determinants of health, including health literacy, using materials available in both English and Spanish. Specific aims are to: (1) assess the prevalence of fall risk among Hispanic and non-Hispanic White adults ≥ 40 years old, (2) determine the prevalence of previously undiagnosed benign paroxysmal positional vertigo (BPPV) in underserved communities, and (3) construct a predictive model of fall risk incorporating auditory, vestibular, and social determinants of health with a focus on falls risk among Spanish-speaking Hispanic adults over age 40.

Methods: Participants from the Harrisonburg, VA community completed the CDC STEADI screener, pure-tone audiometry, tympanometry, vestibular assessments (Dix-Hallpike, vHIT, VEMPs, Rotary Chair), and health literacy/social determinants questionnaires. Recruitment occurred through local community centers, churches, and the university.

Results: Data collection is ongoing and expected to wrap up fall 2025 with a sample size of 80. Preliminary pilot data (N = 14; mean age = 54.6 years) suggest that fall risk prevalence is higher among Hispanic participants compared to non-Hispanic White adults. The CDC STEADI identified 43% of Spanish-speaking Hispanic participants as at risk, > the national average. BPPV prevalence in the Hispanic participants was higher than national estimates (14% vs. 3%). Logistic regression analyses indicated that participants with BPPV were approximately five times more likely to be classified as at risk for falls compared to those without BPPV, and participants with low health literacy had 1.5 times greater odds of being at risk relative to those with adequate health literacy. These findings suggest that both vestibular dysfunction and health literacy are important contributors to fall risk and may be underrecognized in underserved populations.

Conclusions: Early findings suggest that using audiologic measures such as BPPV screenings and social determinants of health such as measuring health literacy, can add predictive value to determining who is at risk of falls. Incorporating audiologic and vestibular assessments improved detection and revealed disparities in risk factors. These preliminary results support larger-scale studies to validate predictive models integrating sensory and social determinants of health.

SU49. The VGluT1-Cre Mouse Targets a Subpopulation of Central Zone Afferents in the Mouse Vestibular Epithelia

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Background: In the vestibular nerve, the temporal precision needed to code rapid head movements is determined by neurons firing at irregular intervals, whereas the ability to detect slow head movements sensitively is determined by neurons firing at regular intervals. The dendritic terminals of irregular afferents are predominantly located within the central zones of the sensory epithelia while the terminals of regular afferents are within the peripheral zones (Fernández et al. 1988; Lysakowski et al. 1995). Because afferent firing patterns are tightly linked to central versus peripheral zones, we tested whether ion channel composition contributes to this diversity by using a Vglut1-Cre driver mouse crossed with an Ai14 TdTomato reporter to selectively label and record from ganglion neurons originating in the central zones of vestibular epithelia. Here we report the peripheral innervation pattern of the fluorescent neurons, their co-expression with Calretinin, and their biophysical properties. This work extends our previous report of central-zone innervation by providing an updated account of their molecular and biophysical features.

Methods: The prevalence of Vglut1-Cre positive (TdTomato positive) neurons was quantified in immunolabeled samples of whole-mount epithelia and cryo-sectioned vestibular ganglia from adult (post-natal day (P)60-P70) mice. We used antibodies against Beta-III tubulin (most somata) and Calretinin (sub-population of central zone somata) to evaluate the overlap between fluorescent somata and other sub-populations. Cross-sectional area of each cell was inferred by the boundaries of the Beta-III tubulin signal. Finally, we recorded using perforated patch-clamping methods from disassociated and cultured ganglion somata at two age ranges (P16±1 days) and adult (P60-P140).

Results: In epithelial whole-mounts, TdTomato positive neurons contacted the central zone of vestibular epithelia with both bouton and calyx terminals. In ganglion sections, TdTomato positive somata were found amongst the medium and large somata and constituted approximately 6% of the total population. Approximately two-thirds of the TdTomato positive somata also co-labeled with the central zone marker Calretinin. Patch-clamp recordings demonstrated that the fluorescent neurons have both transient and intermediate firing patterns. They also responded to simulated synaptic noise with irregular responses, qualitatively support the hypothesis that ion channel properties of central-zone fibers account for irregular firing.

Conclusions: The Vglut1-Cre^{*}Ai14 mouse line effectively targets vestibular afferents, with a bias toward irregularly firing central-zone fibers. The biophysical properties of these neurons support their role in generating irregular firing. Ongoing work is characterizing regional variations in ion channels (KCNQ, HCN, and Kv1) that may underlie differences in quantal versus nonquantal transmission. This line offers a promising tool for testing the functional contributions of central-zone neurons through genetic manipulation.

SU50. PROVIDE: Patient-Centered Care for Vestibular Disorders

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Background: Vestibular disorders extend far beyond dizziness and imbalance. They encompass a spectrum of symptoms, ranging from nausea and a loss of equilibrium to cognitive impairments, depression, and reduced quality of life. Current treatments remain limited in both effectiveness and personalization. The PROVIDE Doctoral Network (<https://provide-dn.eu/>), funded under the Marie Skłodowska-Curie Actions (MSCA) within the Horizon Europe framework, addresses this pressing clinical challenge. The goal of the network is to advance patient-centered care for vestibular loss by integrating various methodologies and expertise across disciplines and bridging scientific research with innovative solutions.

Methods: PROVIDE's research activities are integrated across three complementary work packages (WPs) identifying the underlying etiology (WP2), improving characterization (WP3), and bringing innovative treatments of vestibular loss to home use (WP4). Through an interdisciplinary approach, PROVIDE unites expertise from pre-clinical animal models and organoids to novel diagnostic tools to clinical interventions using haptic biofeedback, neuromodulation, and vestibular implants. In total, 22 partners from 12 countries are involved in the training and scientific development of 10 doctoral candidates.

Results: PROVIDE is generating scientific insights that advance the pathway from identification of the individual vestibular disorder etiology to providing personalized treatment. It is also developing new tools and methods that are freely available to all researchers. In addition, PROVIDE is creating openly accessible educational resources and training materials through an online learning platform as well as opportunities to participate in training events.

Conclusions: By promoting equitable and inclusive practices in training, research, and healthcare, PROVIDE is advancing interdisciplinary vestibular research and contributing to the development of sustainable tools to transform the management of vestibular loss and foster knowledge exchange and outreach beyond the network.

SU51. Effects of Prosthesis Stimulation on Compensatory Saccades During the En-Bloc Head Impulse Test in Vestibular-Impaired Monkeys

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Background: The vestibulo-ocular reflex plays a critical role in gaze stabilization. Patients with vestibular loss cannot maintain gaze on a target during head movements because the eyes move with the head. The head-impulse test (HIT) detects this instability by having patients fixate on a target while the head is rapidly and unpredictably rotated to stimulate the semicircular canals. Patients make two types of compensatory saccades during HIT: covert saccades, which occur during head rotation, and overt saccades, which occur after head movement stops. Patients who acquire covert saccades show improved Dizziness Handicap Inventory scores, higher visual acuity, and reduced oscillopsia, likely because retinal slip is minimized and vision is suppressed during the saccade. Learning to generate covert saccades is therefore important for quality of life. To study the neural basis of these movements, we developed a vestibular-impaired monkey model and confirmed use of a saccadic strategy during en-bloc HIT, in which head and body rotate together.

A vestibular neuroprosthesis has been used to replace missing sensory motion information. Although significant progress has been made in surgical implantation techniques and waveform optimization over the past two decades, functional restoration remains incomplete. Novel approaches are therefore needed to achieve full recovery. As a supplement to current strategies, one approach is to understand how the brain integrates and adapts to artificial stimulation delivered alongside natural neural activity, including residual vestibular function and saccades. In this study, we test whether vestibular prosthesis stimulation facilitates saccades during en-bloc HIT and assess its long-term effects.

Methods: One bilaterally vestibular-impaired monkey was tested during en-bloc HIT at a peak velocity of 100°/s. The right horizontal semicircular canal was stimulated with a vestibular prosthesis delivering biphasic pulses at 250 pps.

Results: Vestibular neuroprosthesis stimulation facilitated leftward saccades. Repeated sessions enhanced performance in both stimulation and no-stimulation conditions, and this effect persisted for several days.

Conclusions: Right-ear stimulation drives activity in the right vestibular nucleus, which in turn enhances activity in the left abducens nucleus through excitatory projections and reduces right abducens nucleus activity via inhibitions. During leftward saccades, the right abducens serves as antagonist whereas the left abducens as agonist. As stimulation depressed antagonist activity and enhanced agonist activity, the balance shifted toward easier saccade initiation. Furthermore, the long-lasting effects suggest that stimulation also induces adaptation or plastic changes in neural circuits that control gaze stabilization.

These findings indicate that vestibular prosthesis stimulation may facilitate recovery by enhancing covert saccade initiation.

SU52. Defining Contributions of the Vestibular Nuclear Complex (VNC) to Vestibular Short Latency Evoked Potentials (VsEPs) of the Rat

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Background: The vestibular system is crucial for posture, gait, and the perception of head and body position in space. Our lab has shown that linear jerk stimulation of irregular vestibular afferents activates central vestibular pathways and is measurable with vestibular-short latency evoked potentials (VsEPs; Holt et. al, 2025). Previous lesion studies in chickens have shown that P2 and later peaks of the VsEP waveform reflect activity of central pathways (Nazareth and Jones, 1997). However, the contribution of the vestibular nuclear complex (VNC) to mammalian VsEP waveform peaks is not well understood. Designer receptors exclusively activated by designer drugs (DREADDs) are genetically engineered receptors used to modulate neuronal activity. These receptors are only activated upon administration of an exogenous ligand. We expressed inhibitory DREADDs in the VNC to initially determine whether the VNC contributes to later VsEP peak(s).

Methods: Sprague-Dawley rats received bilateral VNC injections of an inhibitory DREADD viral vector (AAV2-CAG-hM4Di-P2A-mCherry-WPRE) via stereotaxic surgery. Craniotomies were sealed with dental cement, and a ceramic nut was fixed at Bregma to allow attachment to

the shaker arm for jerk stimulation. Three - 4 weeks after the surgery, VsEPs were recorded across two sessions, at least one week apart. Each session included two blocks of 2,000 jerks (1,000 up, 1,000 down, ~3,300 g/s). During session one, animals received saline (IP) before block one and deschloroclozapine (DCZ; DREADD agonist) before block two. During session two, saline was given prior to both blocks to control for the potential effect of repeated stimulation. VsEP amplitudes and latencies (P1, P2, P3) in each session were analyzed and compared across blocks. To determine which brain regions expressed the fluorescent DREADD receptors, animals were transcardially perfused. Brains were postfixed, cryoprotected, serially sectioned (40 μ m), and imaged.

Results: During the first session, P3 amplitude was significantly reduced in the block following DCZ administration compared to the saline block ($p < 0.05$, paired T-test). There were no significant differences in P1, P2, and P3 amplitudes between blocks in session 2. Administration of DCZ also increased P2 response latencies compared to the saline block in session one ($p < 0.05$). No significant differences were found between the saline blocks in session two. To confirm DREADD expression, we imaged histological sections through the VNC.

Conclusions: Our data show that partially inhibiting rostral VNC activity reduces P3 neuronal synchrony and delays P2 and P3 response times. The changes observed in synchrony and process speed reflect VNCs contribution to later VsEP waves. These results advance the interpretation of VsEPs as a tool for assessing both peripheral and central vestibular function. Future studies should assess the effects of rostro-caudal position and VNC subdivisions to the VsEP waveform.

SU53. Characterization of Auditory and Vestibular Consequences in a Preclinical Single and Repetitive Blast Model

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Background: Blast explosions can inflict significant damage on the auditory and vestibular systems. Acute symptoms following even mild blast exposures can include tinnitus, hearing loss, dizziness, and disorientation, whereas high rates of chronic sensorineural hearing loss and vestibular dysfunction are reported in the military population. In this study, we directly compare outcomes following single vs repetitive exposures to investigate progressive inner ear functional loss caused by low-intensity blasts.

Methods: Brown Norway rats (n=8; 14-18 weeks) were randomized to single-blast (8–12 psi), repetitive blast (10 total blasts, once daily) or sham (no blast). Anesthesia for the blast was achieved with isoflurane supplemented with O₂. Auditory brainstem response (ABR; 4, 8, 16, 24 kHz) and cervical vestibular evoked myogenic potential (cVEMP; 1,8 kHz) amplitude, latency and thresholds are collected at baseline, blast day (BD) 5 and BD10, post-blast day (PBD) 7, PBD14, and PBD 28. Tympanic-membrane integrity was examined prior to electrophysiological recordings. Gait kinematics, including fine movement and posture were analyzed across the groups.

Results: Both single-blast and repetitive-blast groups exhibited a significant shift in ABR and cVEMP thresholds compared with sham controls. The elevation in thresholds was accompanied by changes in amplitudes and latencies consistent with acute auditory and vestibular dysfunction. By PBD28, animals in the single-blast group demonstrated recovery, with ABR and cVEMP thresholds returning toward baseline values. In contrast, the repetitive-blast group exhibited persistent ABR and cVEMP threshold elevations at PBD28 compared to both sham and single-blast animals.

Conclusions: These findings indicate that, while both single and repetitive mild blast exposures disrupt peripheral auditory and vestibular function, recovery diverges across these groups over time. Single-blast animals show transient deficits that are partially resolved within 28 days, whereas repetitive-blast animals exhibit sustained auditory and vestibular dysfunction. This suggests that cumulative blast exposure potentially impairs endogenous functional recovery and produces long-lasting sequelae, highlighting the importance of further investigation of its mechanism.

SU54. Identifying Neurons in the Vestibular Nuclei Responsive to Vestibular Evoked Potentials

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Background: The peripheral vestibular system consists of two otolith organs that detect linear accelerations and three cristae which detect angular accelerations. These sensory organs are innervated by the vestibular ganglion that relays peripheral inputs to the vestibular nuclei (VN) located within the brainstem, as well as to the cerebellum. However, the circuitry and identity of the VN neurons are still largely unknown; specifically, how peripheral vestibular information from the end-organs is relayed and processed centrally is poorly understood. An important functional assessment of the striola of the otolith organs is the initiation of compound action potentials known as vestibular-evoked potentials (VsEP), in response to jerk stimuli to the head. Understanding how the VN respond and process inputs from the striola will provide insight into central processing of vestibular information in general. To identify VN neurons responding to VsEP, we examined the expression of c-Fos, an immediate early gene induced during neuronal activation, following jerk stimulation in mice. To further validate that the observed c-Fos expression is associated with VsEP, we performed the same jerk stimuli on Tmie knockout mutants, in which all hair cells lack mechanotransduction and consequently, VsEP is absent.

Methods: We applied a modified VsEP protocol to stimulate the utricle and saccule in anesthetized adult mice using maximum, repetitive linear jerk stimuli continuously for 20 minutes. Control animals also received anesthesia but were not stimulated. Thirty minutes after the end of stimulations, transcardiac perfusion was performed. Then, the brain was dissected, fixed, and cryopreserved. During processing, coronal cryosections of the brainstem at the level of VN were collected. Multiplex RNAscope was performed using probes for c-Fos, paired with Calbindin-II and Neurofilament for VN identification. In WT animals, comparisons were made between stimulated and unstimulated controls. In Tmie^{-/-} experiments, comparisons were made

between stimulated Tmie^{-/-} mutants against unstimulated Tmie^{-/-} and stimulated Tmie^{+/-} littermates.

Results: Stimulated WT mice showed an increase in c-Fos labeled neurons in caudal medial vestibular nuclei (MVN) and descending vestibular nuclei (DVN), compared to unstimulated controls. This increase in c-Fos expression is consistent with the role of striola in mediating postural control. Congruently, such increase of c-Fos⁺ neurons in DVN of Tmie^{+/-} stimulated controls was strongly reduced in stimulated Tmie^{-/-} mice. By contrast, an increase in c-Fos⁺ neurons in the rostral MVN was observed in both stimulated and unstimulated Tmie^{-/-} animals, compared to Tmie^{+/-} littermates.

Conclusions: Results of the Tmie^{-/-} mutants support the conclusion that the increased c-Fos expression found in caudal MVN and DVN after jerk stimuli is associated with VsEP. Currently, we are investigating whether these c-Fos⁺ neurons are excitatory or inhibitory in nature using known molecular markers. We are also characterizing the upregulated c-Fos expression in rostral MVN of Tmie^{-/-} mutants, which is independent of jerk stimuli.

SU55. Dexamethasone-Eluting Cochlear Implantation Reduces Intracochlear CX3CR1⁺ Cellular Infiltration and Foreign Body Response in Long-Term Follow-Up in Mice

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Background: Cochlear inflammation and foreign body response following cochlear implant (CI) surgery may impact clinical outcomes by contributing to delayed loss of residual acoustic hearing and increasing CI electrode impedance. We have previously reported that a dexamethasone-eluting CI (DexCI) reduces macrophages infiltration and the foreign body response when assessed up to 112 days post-CI in mice and reduces CI impedance values in humans in a similar time-frame. It is not known whether these anti-inflammatory and anti-fibrotic

effects persist at clinically relevant longer-term follow-ups. This study aims to assess the effect of the DexCI in reducing the post-CI foreign body response at longer time-points beyond when dexamethasone elution is anticipated to be diminished.

Methods: 10-12-week-old CX3CR1⁺/GFP Thy1⁺/YFP mice (n=6 per group at each timepoint) on

C57Bl6 background with normal hearing, were implanted with a standard or dexamethasone-eluting mouse CI (standard CI, DexCI, respectively) in the left ear via the round window; with the

right ear being an unoperated control. Cochleae were harvested at 224- or 336-days post-CI

and prepared for immunohistochemistry to assess macrophages (CX3CR1), spiral ganglion neurons (Thy1), fibrotic tissue (α -SMA) and nuclei (DAPI). Subsequent Movat's Pentachrome staining was performed to further characterize cochlear fibrosis and neo-ossification and assess for cochlear trauma. The density of CX3CR1+ macrophages was measured in the scala tympani (ST), Rosenthal's canal (RC) and lateral wall (LW) as well as the spiral ganglion neuron (SGN) density and volume of α -SMA-positive fibrotic tissue in the ST for each cochlear turn.

Results: All cochleae implanted with a Standard CI showed robust cochlear fibrosis and neo-ossification at 224- and 336-days. However, DexCI cochlea showed minimal to no foreign body response, except in 2/6 cases at 224-days where basilar membrane translocation of the CI was identified. CX3CR1+ macrophage density and α -SMA-positive fibrotic tissue was significantly reduced in the ST, LW and RC of the basal turn in the mice implanted with DexCIs when compared with standard CIs at both 224- and 336- days post-CI. The qualitative degree of neo-ossification and fibrosis was most pronounced in cases of translocation in the Standard CI group, with the FBR spanning all three scala in instances of translocation versus being confined to the ST in cases without CI translocation.

Conclusions: DexCI reduced macrophage infiltration and fibrosis relative to Standard CI over the long-term (224- and 336- days post-CI), except in cases where basilar membrane translocation was identified. Further, we observe that insertion trauma in the form of CI translocation may amplify the CI foreign body response. These findings show that in a mouse model, DexCI reduces fibrosis well beyond the expected dexamethasone elution window, suggesting the foreign body response mitigation to be a durable effect long-term.

SU56. Systemic Treatment With Antisense Oligonucleotides Restores Vestibular Function in Usher 1C Mice

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Background: Usher syndrome (USH) is a rare genetic disorder characterized by the multi-sensory loss of hearing, balance, and vision. Approximately 2.5% of USH is caused by mutations in the USH1C gene, encoding the protein harmonin expressed in hair cells. While assistive devices such as cochlear implants help manage hearing loss, no therapies exist to restore vestibular function. Here, we studied the therapeutic effects of antisense oligonucleotides (ASO-29) to treat balance dysfunction in an Ush1c c.216G > A knock-in mouse model of USH1C. The ASO-29 targets the 216A mutation to redirect aberrant Ush1c splicing and promote full-length harmonin expression.

Methods: A single dose of ASO 29 was delivered to USH1C mice by intraperitoneal injection at postnatal day 2 (P2) and aged to 6 months. Vestibular function was assessed in ASO-treated and

untreated USH1C mice and wild-type (WT) littermate controls by measuring the vestibulo-ocular reflex (VOR) responses. Eye movements were recorded using a high-speed eye tracking system. Rotational VOR (rVOR) and translational VOR (tVOR) were measured to assess semicircular canal and otolith function, respectively. Gains, phases, and distortion indexes were calculated by performing a fast Fourier transform (FFT) analysis. General motor behavior was assessed by open-field, rotarod, and balance beam analyses. Hearing function was assessed by auditory-evoked brainstem response (ABR) analyses.

Results: At six months of age, untreated USH1C mutant mice had significantly reduced rVORs compared with WT littermates, indicating severe canal function deficits. TVOR gains remain comparable to WT mice. Additionally, untreated USH1C mice showed circling behavior, and significantly increased latency-to-fall on the rotarod and time-to-traverse the balance beam, and significantly elevated hearing thresholds at all frequencies tested compared with WT littermates. ASO-29-treated USH1C mice showed robust rVOR responses corresponding to 56 to 65% of WT levels across the tested frequency range. ASO-treated USH1C mice also showed improved latency-to-fall on the rotarod and time-to-traverse on the balance beam compared with untreated USH1C littermates. ABR analyses showed improved of hearing thresholds in ASO-treated USH1C mice compared with untreated USH1C mice.

Conclusions: Systemic ASO-29 treatment in USH1C mutant mice led to a significant restoration of vestibular canal function, vestibular behavior, and hearing function. These results demonstrate the therapeutic potential of ASOs to treat balance deficits associated with Usher syndrome. (supported by NIH R01 DC020243-01A1, 1P20GM156708-01) (JH and JL: co-presenter, JL, WZ, HZ co-correspondents)

SU57. Postnatal Role of Jagged1 in the Hair Cells and Supporting Cells of the Vestibular System

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Background: The vestibular system, composed of the utricle, saccule, and semicircular canals, is essential for balance and spatial orientation. Sensory function within this system relies on two types of hair cells (HCs) (type I and type II), which detect head motions and gravity. Supporting cells (SCs) encase HCs, providing structural support and protecting HCs from damage, much like glial cells support and protect neurons in the central nervous system. Jagged1 (Jag1), a Notch ligand, is crucial for inner ear development, guiding progenitor cell formation, while other Notch ligands mediate the differentiation of progenitor cells into HCs and SCs. While Jag1's role in early cochlear and utricular development is well-established, its continued postnatal expression suggests an additional function during maturation. Prior research demonstrated that deleting Jag1 from SCs of the cochlea at birth led to the loss of a SC subpopulation, called Hensen cells, caused a mild hearing loss, and stereocilia deficits in inner HCs (Chrysostomou et al., 2020; Gilels et al., 2022). Previous studies have shown that Jag1 is expressed in the utricle after birth and into adulthood, suggesting its potential involvement in other processes such as postnatal HC addition, maturation, turnover or survival. Here we deleted Jag1 from utricular HCs or SCs at birth to understand its function.

Methods: We generated mouse models to delete Jag1 specifically from HCs or SCs at postnatal day (P) 1 using: Atoh1-CreER:Jag1^{f/f}:Rosa26Tom^{+/f} mice for HC-specific Jag1 deletion, and Plp-CreER:Jag1^{f/f}:Rosa26Tom^{+/f} mice for SC-specific Jag1 deletion. Utricles were immunostained with myosin VIIA to label HCs, SOX2 to label SCs and type II HCs, and Phalloidin to visualize phagosomes and stereocilia bundles. We quantified the number of each cell type at 2 and 4 weeks after the Jag1 deletion using confocal microscopy.

Results: Our preliminary results suggest a decrease in the number of SCs following Jag1 deletion from SCs, suggesting that Jag1 plays a role in maintaining SC survival postnatally. Additionally, when Jag1 was deleted from utricular HCs, we observed an increase in the number of phagosomes that target HCs at both 2 and 4 weeks post-deletion compared to controls. However, our preliminary results showed that there is no significant difference in the number of type I or type II HCs in HC-specific deletion of Jag1. This lack of change may be due to endogenous cellular turnover mechanisms that compensate for the loss of Jag1, preserving the overall HC population. However, a larger sample size and analysis at longer post-deletion periods are needed.

Conclusions: Our findings suggest that Jag1 plays a role in maintaining the survival of SCs in the postnatal utricle. The increase in phagosomes when Jag1 was deleted from HCs also suggests a role for Jag1 in the natural HC turnover process.

SU58. Effects of Tmie Deletion on Vestibular Hair Cells in Mice

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Background: Tmie gene encodes a protein that is crucial for the mechanoelectrical transduction (MET) channel complex in the hair cells in the inner ear (Zhao et al., 2014). In a prior investigation, we characterized the vestibular phenotypes of spinner, a mouse model with a Tmie deletion for human hearing loss DFNB6 (Iriarte Oporto et al., 2025). Vestibulo-ocular reflex (VOR) tests showed that Tmie KO mice exhibited no eye movement responses to head rotation (rotational VOR, rVOR) and substantially reduced responses to head translation (translational VOR, tVOR). Additionally, single vestibular afferent recording showed that substantial reduction in the spontaneous firing rates of both canal and otolith afferents, a decrease in the population of regular canal afferents, and diminished sensitivity of otolith afferents to translation. In this study, we further examined whether loss of vestibular hair cells may underlie the functional deficits we observed.

Methods: Temporal bones were collected from 4 to 7-month-old male and female C57 BL/6 wild-type (WT), Tmie^{+/-}, and Tmie^{-/-} mice. Animals were perfused transcardially with 4% paraformaldehyde, and temporal bones were post-fixed, decalcified in 0.25 M EDTA, and microdissected to isolate cristae and maculae for whole mount processing. Immunolabeling was performed using primary antibodies against Myo7a, Sox2, and OCM. Myo7a specifically labels all hair cells, Sox2 identifies both supporting cells and type II hair cells, and OCM highlights the striolar region of the utricle and saccule and the central region of the cristae. Imaging was

conducted utilizing a Zeiss LSM 880 confocal microscope and image analysis was carried out with Zen Blue software and Fiji/ImageJ.

Results: Preliminary analysis showed no significant differences in overall hair cell density in the saccules and horizontal canals between control and Tmie KO mice. Similarly, OCM-positive cells in the utricle, saccule, and horizontal cristae did not differ between control and Tmie KO mice. Sox2 immunostaining showed increased proportion of Type II hair cells in the central region of the horizontal cristae, and the extrastriolar region of the saccule in Tmie KO mice.

Conclusions: Although preliminary, our morphological analysis indicates that vestibular hair cells are preserved in Tmie KO mice, suggesting that the observed vestibular functional deficits are not attributable to hair cell loss. This contrasts with the auditory system, where Tmie KO mice exhibit clear hair cell degeneration. Ongoing studies are focused on examining the structural integrity of vestibular hair cell hair bundles in Tmie KO mice to further clarify the basis of the functional impairments.

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SU59. (Micro)structure of the Brainstem-Cortical Pathways and Their Relation to Auditory Categorization and Speech-In-Noise Performance

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Background: Successful speech perception requires listeners to bin continuous acoustic information into discrete phonetic categories. Categorization skills vary among listeners; some maintain within-category acoustic information (gradient) while others discard category-irrelevant information (discrete) during perception. Listeners also vary in how consistently they label speech sounds and more gradient/consistent labeling has been linked with better speech-in-noise (SIN) perception. How gross brain anatomy and microstructure (i.e., white matter tract density) of the brain's major speech-language and auditory pathways relates to individual differences in speech categorization and SIN processing has not been explored.

Methods: We assessed individual differences in categorization and SIN comprehension via phoneme labeling and QuickSIN tasks. Diffusion-weighted imaging (DWI) with probabilistic tractography estimated axonal density (quantitative anisotropy – QA) of bilateral arcuate fasciculi and the acoustic radiations. Anatomical segmentation provided estimates of surface area, gray matter volume, and thickness of important frontotemporal auditory-linguistic brain areas (Broca's area) and the midbrain.

Results: Behaviorally, we found QuickSIN scores were correlated with changes in the slope of listeners' identification curves suggesting a more stable categorization listening strategy yielded better (lower) SIN thresholds. DWI data showed that listeners with greater white matter density in bilateral arcuate fasciculus and the left acoustic radiation performed better on the QuickSIN. Further, morphometric results revealed more perceptually consistent listeners had reduced surface area and gray matter volume in left Broca's area. Behaviorally, we found QuickSIN scores were correlated with changes in the slope of listeners' identification curves suggesting a more stable categorization listening strategy yielded better (lower) SIN thresholds. DWI data

showed that listeners with greater white matter density in bilateral arcuate fasciculus and the left acoustic radiation performed better on the QuickSIN. Further, morphometric results revealed more perceptually consistent listeners had reduced surface area and gray matter volume in left Broca's area.

Conclusions: These findings support the notion that individual differences in phonetic categorization account for variance in SIN comprehension and are at least partially explained by differences in anatomical properties of the auditory-linguistic brain. Closer association between structures of the left cerebral hemisphere (surface area, gray matter volume) and behavioral response consistency in phoneme labeling suggests listeners with more stable phonetic perception make more efficient use of frontal language areas. Higher density in the acoustic radiations further suggests that the subcortical auditory pathways also contribute to better SIN understanding. This may involve afferent but also corticofugal efferent pathways.

SU60. Subcortical Speech Coding Predicts Perceptual Consistency in Phoneme Categorization and Improved Speech-in-Noise Perception

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Background: Listeners discretize the speech signal by assigning sounds to phonetic categories, though there is variability between individuals in how they accomplish categorization. Retaining more within-category acoustic information (gradiency) and having more consistent perception of sounds may be advantageous for understanding speech-in-noise (SIN). It is unclear how different levels of neural processing in the auditory system reflect these perceptual differences. In particular, whether subcortical speech-evoked responses drive perceptual consistency and gradiency in acoustic- mapping has not been thoroughly explored.

Methods: We recorded brainstem frequency-following responses (FFRs) while listeners actively labeled vowels along an acoustic-phonetic continuum using a visual analog scale. We measured individual differences in gradiency and consistency via phoneme labeling and SIN comprehension via the QuickSIN. We computed intertrial consistency of FFRs to index the stability of listeners' neural speech representation across stimulus presentations. We also assessed how faithfully midbrain responses represented stimulus acoustics using representational dissimilarity matrices (RDMs) computed across all token pairs. Neural RDMs were then compared with acoustic RDMs to estimate neuro-acoustic correlation (NAC) and thus whether FFRs carried gradient vs. discrete information of the speech signal.

Results: Greater behavioral consistency during phoneme labeling was correlated with improved SIN scores. Neurally, we found listeners with greater behavioral consistency also had more consistent subcortical representations of speech sounds in their FFRs. Likewise, more consistent listeners and more gradient listeners had higher NAC in subcortical RDMs, suggesting a more accurate and detailed encoding of the speech continuum.

Conclusions: Our findings reveal important benefits of perceptual consistency (and to a lesser extent gradiency) to several domains of speech perception. We find perceptual consistency is driven by more faithful stimulus feature encoding at a surprisingly early, subcortical level of the auditory system. More consistent sensory processing could provide a more stable readout of the

speech signal to higher cortical brain areas. This could in turn confer advantageous to later perceptual processes downstream, including understanding speech in noise.

SU61. Comparison of Speech in Noise Processing in Hearing Impaired Populations Using [15O]Water Pet

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Background: Difficulty with speech in noisy real-world environments remains a primary complaint in hearing impaired (HI) listeners despite successful use of amplification devices. Research in normal hearing listeners indicates that auditory cortex is active while abstracting speech objects from noise and provides input to fronto-temporal networks for further perceptual, attentional, and semantic analysis. We want to understand whether these are the same neural mechanisms across hearing impaired listeners with different devices (CI and hearing aid) and configurations, and how these mechanisms relate to successful hearing outcomes.

Previously we demonstrated in a proof-of-concept study that we could robustly and reliably measure brain [15O]Water positron emission tomography (PET) blood flow activity to speech in noise in cochlear implant users at a single subject level. We observed activity in a fronto-temporal network to speech in noise in each single subject. Here we expand our investigation to include a broad range of hearing impairment (CI and hearing aid users) and device configurations to capture variability that may be different to that of a matched control population.

Methods: We measured brain blood flow using [15O]Water PET in a group of 44 cochlear implant subjects, 20 hearing aid users, and 20 age-matched normal hearing controls while they performed a word-in-noise identification or noise-control task (matched on RMS sound level). Six runs were performed for each condition. On a given 3-min run for speech in noise (+15 dB SNR), word tokens were presented in multi-talker babble, and at random intervals subjects were prompted to perform a 4-alternative forced choice task. The control condition was a noise level detection task matched for response demands. PET data were analyzed in SPM12 using a flexible factorial model.

Results: We found robust activations in single subjects for the contrast speech in noise vs control noise in auditory cortex and inferior frontal cortex. Group level activation regions of interest in auditory cortex and inferior frontal cortex were significant, along with activations across a network of areas involved in language processing ($p < 0.05$, corrected). Group comparisons highlighted differences in the involvement of frontal cortex which may relate to the existence of acoustic hearing. A regression analysis including speech in noise task performance indicated the involvement of brain regions for attention and object analysis.

Conclusions: Our results show that speech in noise processing in different hearing impaired populations may depend on the availability of residual acoustic hearing, and the use of brain networks for language similar to that of the normal hearing population.

SU62. Intelligibility Modulates Early Attentional Filtering of Competing Speech

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Background: The intelligibility of competing speech influences how it interferes with perception of target speech. However, most previous studies have failed to equate local spectrotemporal statistics; thus, observed differences in performance may reflect differences in low-level acoustic features. Also, few measured underlying neural responses. Here, listeners performed a selective attention task, detecting target color words amidst competing, spectrotemporally matched intelligible or unintelligible maskers. We measured simultaneous electroencephalography (EEG) to gain insight into underlying mechanisms.

Methods: Twenty different normal-hearing listeners participated in each of two Experiments. Listeners were asked to attend to a target stream comprising color and object words spoken by a male talker, while ignoring a masker stream spoken by the same or a different male talker. Target words were randomly presented in time, while the masker stream was ongoing. The masker stream comprised either words drawn from the same set (Words masker), or a spectrotemporally scrambled version (Scrambled masker). Target and masker streams were also filtered in adjacent non-overlapping frequency bands to reduce energetic masking. Listeners were asked to detect target color words by pressing a button. We simultaneously measured evoked responses using EEG. In Experiment 1, the scrambling procedure resulted in a flat amplitude envelope masker. Experiment 2 served as a control, where the Scrambled masker stream was amplitude modulated with a Words masker envelope to match isochronous acoustic onsets.

We computed hit rates (responses to color words in the target stream), within-stream false alarm rates (responses to object words in the target stream), and between-stream false alarm rates (responses to color words in the masker stream in the Words condition). From the EEG, we computed the amplitude of onset sensory P1-N1 responses and P300 target recognition responses.

Results: Results replicated across experiments. A Scrambled masker yielded higher hit rates and lower within-stream false alarm rates than a Words masker. Participants performed better with a different talker than a same talker masker, but only with a Words masker. Between-stream false alarm rates with a same talker Words masker were significantly higher than with a different talker. Evoked responses mirrored behavioral performance. Onset (P1-N1) responses evoked by target color words were larger for a Scrambled masker than a Words masker. This effect was larger when the target and masker were spoken by the same talker. Target recognition (P300) was greater for target color than object words, but did not vary with masker type or talker.

Conclusions: Our results demonstrate that auditory attention is more strongly disrupted by intelligible speech than by unintelligible speech, even when their spectrotemporal properties, and thus the degree of energetic masking, are closely matched. Furthermore, intelligibility and talker differences were reflected in early sensory (P1–N1) responses, but not target recognition (P300) responses, implicating an early-latency attentional filtering mechanism.

SU63. Evaluation of Aided Speech Intelligibility, Listening Effort, and Subjective Sound Quality With Real-Time Deep Neural Network-Based Noise Reduction

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Background: Hearing loss affects many aspects of daily life, yet deficits in speech understanding are especially detrimental to professional productivity, social engagement, and quality of life. As a result, improvement in speech intelligibility, notably in noisy environments, is a primary goal of hearing aid design. Strategies to improve speech understanding include use of directional microphones, and signal processing to emphasize speech-content and reduce noise. Despite these innovations, hearing aid users still frequently report difficulty hearing speech in noise. Recent advancements in computational hardware and deep learning have now made it possible to include a real-time deep neural network (DNN) noise reduction system within the physical form factor of commercial hearing aids. Initial tests of the DNN noise reduction showed up to 10 dB SNR improvement for speech in noise based on acoustic measures (Raufer et al., 2024), and behavioral results show significant improvement in speech intelligibility and reduction in listening effort (Wright et al., 2024).

Methods: Here, to further evaluate this new technology, behavioral measures in the presence of speech-shaped noise tested speech intelligibility, listening effort, and subjective measures of sound quality for the DNN-capable device (with DNN-on and DNN-off), as well as comparable commercial devices from other manufacturers. Participants (N=29) had documented hearing loss and were experienced hearing aid users. The intelligibility task used materials adapted from the coordinate response matrix (CRM; Bolia et al., 2000), with sentences presented in the free field from a range of target-azimuth locations. Listening effort was measured using the adaptive categorical listening effort scaling task (ACALES; Krueger et al., 2017). The subjective listening task presented a 4-minute two-talker conversation, followed by a questionnaire assessing elements of speech clarity, naturalness, loudness, and separation from noise.

Results: Results from the CRM experiment showed significantly higher accuracy by listeners with the DNN-on device configuration compared to DNN-off, notably for target locations at the periphery (+/- 120°) with improvement in accuracy up to 30%. Participant ratings of listening effort were significantly lower for comparable SNRs (-2.9 dB) for the DNN-on configuration compared to DNN-off. Finally, subjective measures of speech quality were significantly better with the DNN for measures of clarity, naturalness, separation from noise, and overall satisfaction compared to alternate devices.

Conclusions: The DNN-capable device tested in this experiment represents the next technological advancement in the search for optimal listener performance by providing real-time noise reduction and subsequent improvement in speech understanding. Quantitative results consistently showed better performance with the DNN-on, as measured by speech intelligibility in the CRM task and reduced listening effort in the ACALES task, without compromising the subjective sound quality provided by the device. Future studies on device-performance in multi-talker environments and real-life listening situations will need to be done to further evaluate this new technology.

SU64. Effects of Listening Effort Demands on Cortical Tracking of Attended and Ignored Narrative Speech

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Background: Aging and hearing loss are associated with increased prevalence of speech perception difficulties in the presence of background noise. To maintain high comprehension performance in such challenging situations, listeners tend to exert greater amounts of cognitive resources, such as selective attention and context-based filling-in and predictive processes, which are collectively referred to as listening effort (LE).

While LE is increasingly recognized as an important factor in speech perception, it is relatively unknown how changes in LE manifest at the level of acoustic, linguistic, and semantic neural representations of speech. Multiple studies have previously reported that aging is associated with amplified cortical representations of continuous speech (e.g., Presacco et al., 2016; Decruy et al., 2019, Mesik et al., 2021), with increases in reliance on LE with age being one of the hypothesized causes of these changes. However, as LE was not directly manipulated in these studies, the extent to which cortical tracking of different speech features is affected by LE remains unclear.

Methods: In the present, ongoing study, we test whether varying the LE demands in a speech comprehension task modulates cortical processing of speech using a large sample of participants (target $n = 60$) spanning a wide age range (18-80 years). While undergoing EEG recordings, participants listen to pairs of audiobooks narrated by different male speakers, instructed to attend to one of the stories and ignore the other. On a trial-by-trial basis (i.e., every ~1 min of the story), LE demands are manipulated by pitch-shifting the ignored story such that the median F0 of the narrator either matches that of the target narrator, making them more difficult to distinguish (higher LE), or they differ by 4 semitones, making them easier to separate (lower LE). Following each trial, participants respond to several comprehension questions about the target story and rate their subjective LE.

Results: The EEG data from each LE condition are analyzed using temporal response function (TRF) analyses, which are used to decompose the EEG signal into contributions from the processing of attended and ignored speech at multiple levels of the processing hierarchy (acoustic, linguistic, and semantic). Effects of LE demands on cortical tracking of speech are evaluated by comparing the prediction accuracies of TRF models from each of the two conditions, and by directly comparing pairs of TRFs for the corresponding speech features between the LE conditions. The outcomes of these analyses will be used to explore how these TRF measures are related to age and cognitive status of the participants (working memory capacity, attentional inhibition, and fluid intelligence).

Conclusions: The results will help elucidate how recruitment of additional cognitive resources in effortful listening shapes cortical processing of speech at multiple levels of the speech processing hierarchy [Supported by NIH grant R21DC020788].

SU65. A Novel Algorithm for Estimating Psychometric Functions in the Matrix Speech-In-Noise Test With Automatic Speech Recognition

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Background: The matrix speech-in-noise test was first developed in Sweden in the early 1980s and is now available in more than 15 languages. Each sentence consists of five words with a fixed syntax: name, verb, number, adjective, and noun. Test material is organized into lists of 10 sentences, each containing unique words. The primary goal is to determine the signal-to-noise ratio (SNR) at which a listener achieves a predefined level of speech intelligibility, typically 50% of words correctly identified. Traditionally, two lists (20 sentences) are required to obtain this threshold. Here, we present a novel adaptive algorithm that uses all SNR–intelligibility data to estimate the full psychometric function. In addition, participant responses are scored automatically using speech recognition, allowing the test to be performed without a tester.

Methods: The procedure consists of three iterative phases. Phase 1 begins with an SNR where no words are recognized (0% intelligibility). The same sentence is then repeated with SNRs increased in 2 dB steps until 100% intelligibility is reached. All SNR–intelligibility pairs are stored, and a sigmoid psychometric function is fitted. From this curve, SNRs corresponding to 20%, 40%, 60%, and 80% intelligibility are extracted and used in Phase 2 with four new unique sentences. The resulting data are combined with Phase 1 to refine the psychometric function. Phase 3 repeats this procedure with updated SNRs and additional new sentences. The final psychometric function incorporates all data, enabling estimation of SNR thresholds at any intelligibility level.

Results: The new method was evaluated in 20 native Swedish speakers with normal hearing. Performance was compared between the classical algorithm (20 sentences, threshold at 50% intelligibility) and the new adaptive algorithm. The adaptive approach typically required fewer than 15 sentences to estimate the full psychometric function, compared with 20 in the classical method. Threshold SNRs at 50% intelligibility differed by < 1 dB between the two approaches. Automatic speech recognition, implemented with OpenAI’s Whisper, achieved nearly perfect accuracy in scoring participant responses.

Conclusions: The proposed adaptive scoring algorithm for the matrix test yields faster results while providing the entire psychometric function, rather than a single threshold value. The 50% SNR thresholds were nearly identical to those obtained with the classical method, confirming its validity. Automatic speech recognition proved highly reliable, enabling fully autonomous administration of the test without a human tester.

SU66. The Consistency of Spectral Contrast and Auditory Enhancement Effects at Higher Frequencies in Speech Perception

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Background: Perception of any sound is influenced by the spectrum of its preceding context. In spectral contrast effects (SCEs), a spectral peak in the preceding context stimulus promotes

perception of spectrally adjacent information in the target sound. In auditory enhancement effects (EEs), a spectral notch in the preceding context stimulus promotes perception of that same spectral region in the target sound (i.e. pop-out effect). SCEs and EEs have long been studied in isolation without much examination of a potential link between them. Stilp and Winn (2025 ARO) reported strong consistency in SCE and EE magnitudes affecting normal-hearing (NH) listeners' categorization of mid-frequency /d/-/g/ targets ($r = 0.76$). However, this consistency did not extend to perception of noise-vocoded speech ($r = 0.05$), possibly due to reduced access to fine spectral details distinguishing /d/ from /g/. Here we assessed the consistency of SCEs and EEs in wider-band, higher-frequency speech cues that distinguish /s/ (as in "seat") from /ʃ/ (as in "sheet"). We predicted the magnitudes of SCEs and EEs would be consistent in how they affected listeners' categorization of spectrally intact and degraded speech. We also predicted that spectral smearing would broaden spectral peaks (increasing SCEs) and make spectral notches less shallow (decreasing EEs).

Methods: Nineteen NH hearing listeners categorized target words from a 10-step continuum ranging from "seat" to "sheet". The target word was always preceded by a context sentence filtered to elicit SCEs and EEs. To elicit SCEs, a bandpass filter amplified energy from 2200-4500 Hz or 4500-8200 Hz to facilitate contrastive perception of "seat" and "sheet", respectively. A bandstop filter attenuated energy in these same frequency regions to elicit EEs.

Results: Both SCEs (more high-frequency "seat" responses when the context sentence had the lower-frequency range amplified) and EEs (more "seat" responses when the context sentence had the higher-frequency range attenuated) influenced perception of both spectrally intact and degraded speech. Data showed consistency among SCE and EE effect magnitudes in spectrally intact speech ($r = 0.45$) but not degraded speech ($r = 0.19$), showing that spectral degradation disrupts the symmetry between these auditory effects even when the target does not demand fine resolution. We observed larger SCEs than EEs in noise-vocoded speech ($p < .001$), consistent with predictions based on spectral smearing.

Conclusions: The consistency of EE and SCE magnitudes in NH listeners' speech perception extends to higher-frequency speech contrasts, but is disrupted with spectral degradation, indicating another fundamental aspect of hearing that is likely affected by CI processing.

SU67. Simulated Peripheral Adaptation Accounts for Spectral Context Effects in Speech Perception

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Background: Perception of any sound is influenced by the spectrum of its preceding context. In spectral contrast effects, a spectral peak in the preceding context stimulus promotes perception of spectrally adjacent information in the subsequent target sound. For example, when perceiving a sound that could be "da" or "ga", a precursor context sentence with prominent energy in low-F3 frequencies (1700-2700 Hz) encourages perception of the high-F3 target "da"; a context sentence with prominent energy in high-F3 frequencies (2700-3700 Hz) encourages perception of the low-F3 target "ga" (Stilp and Assgari, 2017). Here, we used computational modeling to evaluate the hypothesis that adaptation of auditory nerve fibers underlie these perceptual shifts.

Methods: Neural responses to stimuli from Stilp and Assgari (2017) were simulated using a model of the auditory nerve that takes peripheral nonlinearities into account (Zilany et al., (2014). Center frequencies of high-spontaneous-rate fibers spanned 2300-3000 Hz to cover F3 regions of interest. Average neural rates were calculated over a 50-ms window that began at the start of the formant transition. Then, rates were normalized by subtracting the mean of the population response to obtain the population-response shape. Following Maxwell et al. (2020), a decision variable was implemented for the model to mimic behavioral responses. On each trial, if the shape of the model's population response to the target syllable more closely resembled its response to the "da" endpoint of the target continuum (smaller Mahalanobis distance), the model selected a "da" response, and vice versa for "ga". This process was repeated 100 times to yield percent correct for each context sentence/target syllable pair.

Results: Simulated neural responses captured essential characteristics of listener data from Stilp and Assgari (2017). The model's "ga" responses monotonically increased as the frequency of F3 onset in the target syllable decreased (became more "ga"-like). Critically, neural responses showed evidence of a spectral contrast effect: more high-F3 "da" responses followed precursor sentences with amplified low-F3 frequencies, and more low-F3 "ga" responses followed sentences with amplified high-F3 frequencies.

Conclusions: Modeling results supported the hypothesis that neural adaptation in the auditory periphery can explain spectral contrast effects in speech perception. Shifts in simulated neural responses accounted for shifts in behavioral responses to the same stimuli. Future work will explore additional experimental paradigms and contributions by later stages of neural processing.

SU68. Subcortical Responses to Connected Speech: The Role of Language Experience

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Background: Prior studies have shown that both long-term language experience and short-term training can enhance subcortical auditory encoding of speech sounds. However, these investigations often rely on frequency-following responses elicited by repetitive, isolated speech stimuli (e.g., single syllables), which do not reflect the dynamic and continuous nature of everyday speech. The present study aims to examine the extent to which language experience influences subcortical processing of connected speech.

Methods: Participants included four groups of normal-hearing adults with varying levels of Mandarin Chinese proficiency: native English speakers with no exposure to tonal languages (n = 11), beginner-level Mandarin Chinese learners (n = 21), advanced Mandarin Chinese learners (n = 14), and native Mandarin Chinese speakers (n = 20). They listened to synthesized Mandarin sentences, each composed of four syllables, with an additional one to three syllables appended at the end of each trial. During the task, they engaged in cyclic syllable counting from 1 to 4 and reported the final count. To assess subcortical auditory processing, temporal response functions (TRFs) were used to model EEG responses using speech features estimated from auditory nerve models. TRF metrics—including model prediction accuracy, peak latency, and peak amplitude—were compared across groups to evaluate the influence of language experience on subcortical encoding of connected speech.

Results: TRFs across all groups showed a prominent peak around 8 ms, resembling the wave V of auditory brainstem responses, indicating subcortical involvement in processing connected speech. No significant group differences were observed in the latencies or amplitudes of this wave-V-like peak, nor in the model prediction accuracy.

Conclusions: These preliminary findings did not provide evidence for an effect of language experience on subcortical processing of connected speech. Further research is warranted to examine subcortical responses to more naturalistic, continuous speech stimuli.

SU69. Effect of Reverberation and Interruptions on Listening Effort

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Background: Everyday listening often occurs in complex environments that include distractions and distortions. Prior work on factors that disrupt spatial selective attention have been conducted in anechoic settings (Liang et al., 2023), but realistic settings involve reverberation that would affect the clarity of both the target signal and the distractors. Although reverberation increases difficulty of target perception, it could also reduce salience of distractors; furthermore, listeners can adapt to stable reverberation in the environment. The impact of reverberation on disrupted auditory attention is therefore unknown. This study tested whether reverberation amplifies or reduces the disruptive impact of interrupters on recall of a target, and whether pupil dilation can index these effects.

Methods: Participants (N=60, normal hearing) heard two competing, temporally interleaved streams of syllables with and without interruptions. Both streams consisted of syllables from the same male talker; they differed only in their location, with one stream spatialized to 30° left and the other to 30° right of center. All syllables and interrupters were spatialized using binaural room impulse responses (BRIRs).

At the start of each trial, a spatialized /ba/ cued the listener to the target stream. Streams comprised 4 syllables randomly drawn with replacement from [/ba/, /da/, /ga/]. Syllable onsets were 600 ms apart; the target began 300 ms before the distractor. At the end of the trial, subjects reported the target syllables in order. In 50% of trials, a novel interrupter occurred 125ms before the onset of the 2nd target syllable. Syllable recall accuracy was compared across conditions. The time course of pupil diameter was baseline-corrected, z-scored, and analyzed using cluster-based permutation tests; peak pupil size latency and amplitude were extracted as summary measures.

Results: Reverberation and interruption each significantly reduced syllable recall accuracy, with the strongest effects for syllables after the interrupter. Interrupters decreased accuracy by ~8%, and reverberation by ~6%. Pupil time courses showed significant clusters of increased dilation for interrupted trials in both environments. Peak amplitude analyses revealed larger dilation for interrupted versus uninterrupted trials, with a marginal effect of reverberation and a significant interaction: The reverberant, uninterrupted condition elicited the smallest peak amplitude despite

being behaviorally most difficult. Peak latency occurred earlier under both reverberation and interruption, with additive effects but no interaction.

Conclusions: Unexpected interrupters reliably increase cognitive effort, amplified under reverberation. Uninterrupted reverberation produced both the lowest accuracy and smallest pupil response, which may reflect reduced salience of syllable onsets and energetic masking from reverberant tails. These findings extend adaptation theories by demonstrating that although listeners may perceptually adjust to reverberation, working memory and attentional demands remain vulnerable to interference.

SU70. Optimized Feature Gains Explain and Predict Successes and Failures of Human Selective Listening

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Background: Attention enables communication in settings with multiple talkers, allowing us to select sources of interest based on their features. Decades of research have left two gaps in our understanding of feature-based attention. First, humans succeed at attentional selection in some conditions but fail in others, for reasons that remain unclear. Second, neurophysiology experiments implicate multiplicative gains in selective attention, but it remains unclear whether such gains are sufficient to account for real-world attention-driven behavior. To address these gaps, we optimized an artificial neural network with stimulus-computable feature-based gains for the task of recognizing a cued talker’s speech, using binaural audio input (a “cocktail party” setting).

Methods: We optimized a deep neural network to report words spoken by a cued talker in a multi-source mixture. The model received an excerpt of the cued talker’s speech in isolation, followed by a mixture of a different excerpt of the cued talker with other talkers and/or noise sources. Audio was spatialized within simulated reverberant rooms using human head-related transfer functions. Attentional gains were implemented via learnable logistic functions operating on the time-averaged model activations of the cue excerpt. The activations of the mixture were then multiplied by these gains. Gains could be high for features of the cue, and low for uncued features, as determined by parameters optimized to maximize correct recognition. Task performance was measured across a diverse set of diotic and spatial listening conditions for both models and human participants to quantify human-model behavioral alignment.

Results: The model successfully learned to use both spatial and vocal timbre cues to solve the word recognition task. Though not fit to human data, the model matched human performance, exhibiting selection based both on voice qualities and spatial location. The model also predicted novel attentional effects that we confirmed in human experiments. Finally, the model’s internal representations exhibited signatures of “late selection” like those seen in human auditory cortex.

Conclusions: We provide a framework to quantitatively model feature-based auditory attention by optimizing a deep neural network to perform an attentional word recognition task. The results suggest that human-like attentional strategies naturally arise from optimization of feature gains for selective listening, offering a normative account of the mechanisms and limitations of auditory attention.

SU71. EEG-Based Neurofeedback Training of Auditory Selective Attention: Toward Clinical Translation Across Languages and Listening Conditions

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Background: Speech comprehension in noisy environments presents significant cognitive challenges, particularly for individuals with hearing impairments. While conventional hearing aids amplify sound, they often fail to improve listening in noisy settings, underscoring the importance of central auditory processing. Auditory selective attention modulates neural representations to enhance target signals over background noise, and individual variations in this ability correlate with speech-in-noise performance. Previous neurofeedback training (NFT), designed to enhance this attentional modulation, improved word-in-noise perception in normal-hearing English speakers. Building on this, the present work investigates two critical extensions for a step forward to a better clinical viability: 1) whether the training benefits generalize to speakers of a different language (Korean) and to more complex sentence-in-noise (SiN) tasks, and 2) whether the training is effective in a diotic condition, where the target and masker sounds are co-located, which is more representative of real-world listening and a crucial step for application in hearing-impaired populations.

Methods: Two groups of normal-hearing participants were recruited: ten Korean speakers and eight English speakers. All participants underwent weekly NFT sessions for three to four weeks. During training, participants attended to one of two simultaneous speech streams ("Up" with a female voice vs. "Down" with a male voice), presented either dichotically (to the Korean group) or diotically (to the English group). The target speech was visually indicated by coins placed above or below a center line. Based on the attentional state, decoded from single-trial EEG, a rocket icon moved up or down, either collecting or missing the coins. To evaluate the efficacy of training, the strength of attentional modulation and SiN perception were assessed before and after the training period. The strength of attentional modulation was measured by the magnitude of entrainment of the EEG to the attended vs. unattended speech envelope. SiN was assessed using sentences presented against multi-talker babble at varying signal-to-noise ratios (SNRs) and spatial conditions.

Results: Following the training, both studies demonstrated a trend of strengthened attentional modulation. A significant increase was observed in the magnitude of entrainment for the English-speaking group in the diotic condition (Cohen's $d=1.19$, $p=0.012$), with a similar positive trend noted for the Korean-speaking group (Cohen's $d=0.97$, $p=0.091$). Correspondingly, SiN performance showed trends of improvement when the two groups were combined (Cohen's $d=0.76$, $p=0.064$), particularly under challenging SNR conditions.

Conclusions: These complementary preliminary findings demonstrate the potential of this NFT paradigm to enhance sentence-based speech-in-noise perception across different languages and in a clinically relevant diotic listening condition. Further research involving larger populations, including individuals with hearing loss, will validate these results and advance the development of evidence-based rehabilitation strategies to improve communication in noisy environments.

SU72. Neural Correlates of One-Shot and Generalized Recognition Learning of Degraded Speech Signals

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Background: Separating neural correlates of conscious perception from sensory processing is a fundamental goal of cognitive neuroscience. Auditory stimuli that can help dissociate such neural correlates include degraded speech signals such as sine-wave speech (SWS) and noise-vocoded speech. Classic tasks using such stimuli often use "one-shot learning", utilizing the fact that while naive listeners hear SWS as noise or whistles, a single exposure to corresponding original speech (OS) can render SWS word-like. This permits the comparison of different percepts (before and after one-shot learning) despite identical stimuli, effectively separating stimulus processing from conscious perception. However, few studies have examined the neural plasticity associated with such learning or how such effects change across longer timescales (e.g. the course of a one-shot learning SWS experiment).

Methods: 19 participants listened to 60 pre-selected SWS tokens or phonetically similar foils, each taken from a previously curated dataset. Each subject was asked to listen to SWS both before and after hearing corresponding OS and in each case to select the word they perceived in a two-alternative forced-choice (target word or phonetically similar foil). Electroencephalography (EEG) was measured throughout and binned based on whether the word occurred before or after OS (i.e. pre- versus post-OS) and whether the word occurred early or late (i.e., in the first or second half of the experiment, respectively).

Results: Listeners were more accurate at recognizing target words after versus before OS. However, this effect was weakened as the experiment progressed, reflecting the fact that participants' accuracy on pre-OS SWS recognition also increased as the experiment progressed, suggesting generalized SWS learning. Slow cortical responses showed a one-shot learning effect at posterocentral electrodes after 500 ms as well as above-chance pre- versus post-OS decoding, particularly between ~200 and 600 ms post-stimulus. Reflecting generalized learning of SWS, this decoding effect weakened as the experiment progressed. However, this early-versus-late effect was only significant in the alpha band, consistent with time-frequency analysis showing significant early-versus-late differences in the alpha and beta bands from ~50-350 ms after stimulation.

Conclusions: Consistent with previous studies, our results show that listeners can both utilize immediately preceding context to help them recognize otherwise unintelligible SWS tokens, and that they can learn to perceive SWS more generally (i.e., without cues) on a relatively slower timescale, perhaps engaging a novel, learned listening mode for SWS. The neural plasticity underlying both effects is reflected in both the slow cortical potential and higher-frequency (e.g., alpha- and beta-band) EEG signals. Such longer-term learning effects should be considered in one-shot learning experiments.

SU73. Predicting Speech-In-Noise Performance and Noise-Reduction Outcomes With Electrophysiological and Subjective Measures

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Background: Individuals with similar clinical hearing profiles have varying tolerance to background noise, which is an inherent individual trait that may affect their reaction to noise-reduction (NR) processing commonly implemented in digital hearing aids. The present study evaluated such individual characteristics using electrophysiological measures and subjective noise-tolerance profiles to examine their relationship with speech-in-noise performance and NR outcomes.

Methods: The neural signal-to-noise ratio (SNR), an amplitude ratio between auditory evoked responses to target speech and noise onset, was calculated for each individual as a cortical index of noise tolerance from a cohort of 42 individuals with normal hearing. Further, noise-tolerance profiles were derived using k-means cluster analysis to assign participants into distinct subgroups based on their performance on a noise-tolerance domain test, which evaluates the relative importance of three criteria, including noise annoyance, speech interference, and listening effort, in judgment of noise tolerance.

Results: The neural SNR significantly correlated with speech-in-noise performance and NR outcomes with varying processing strengths. However, noise-tolerance profiles did not show any differences in such outcomes among different clusters of participants. The neural SNR and noise-tolerance profiles also did not show any meaningful relationships.

Conclusions: While the neural SNR showed a robust relationship with speech-in-noise performance and NR outcomes, and subjective noise-tolerance profiles were comparatively less sensitive, subjective measures remain one of the most accessible options in the clinic. Thus, we must continue to investigate ways to incorporate both electrophysiological and subjective measures to understand better individual characteristics that determine their noise tolerance levels and hearing outcomes.

SU74. Modeling the Effects of Temporal Incoherence Within Speech on Sentence Intelligibility

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Background: Temporal coherence—the synchronized timing of acoustic events across frequency channels—is considered a core cue in auditory scene analysis, supporting the perceptual grouping of components from a common source. While its importance has been demonstrated extensively with synthetic and non-speech stimuli, its role in speech perception remains unclear. In particular, introducing temporal incoherence into speech by desynchronizing frequency bands does not appear to make it harder to segregate a target speaker from a competing speaker than when listening to speech in steady noise (Lee and Oxenham, 2024, J. Acoust. Soc. Am. 156, 3285–3297). It is unknown how current computational models of speech intelligibility handle speech whose onset of the frequency bands are asynchronized, or whether their predictions would depend solely on the type of background masker. This study compared

model predictions to existing behavioral data to provide new insights into the relationship between model mechanisms and human performance.

Methods: We employed the sCASP model of speech intelligibility (Relaño-Iborra et al., 2019, J. Acoust. Soc. Am. 146, 3306–3317) to predict the behavioral results of Lee and Oxenham (2024). In that study, 25 normal-hearing listeners heard speech filtered into eight contiguous frequency bands and temporal coherence was disrupted by introducing delays between bands. The target speech was presented with either speech-shaped noise or a single competing talker as the masker. Both target and masker could be temporally coherent or incoherent, with the degree of incoherence varying across conditions. The sCASP simulated peripheral auditory processing and generated speech intelligibility predictions by correlating the modulation spectrograms of the clean target speech and the degraded stimulus.

Results: The model predicted a decrease in intelligibility as across-band delays increased, consistent with the general behavioral trend. However, sCASP overestimated the severity of this decline, particularly at larger delays. The model also predicted strong masker type effects, with greater degradation in the speech-in-speech condition. By contrast, human listeners showed relatively similar performance across masker types.

Conclusions: The study examined how temporal coherence affects intelligibility of speech in complex auditory environments, employing a well-established model of speech intelligibility (sCASP) to interpret behavioral data. Our preliminary results reveal mismatches between model predictions and human performance, highlighting limitations in current modeling approaches. These findings provide insights into the mechanisms underlying auditory scene analysis with speech and point to directions for further model development.

SU75. Neural Correlates of Spectro-Temporal Modulation Sensitivity

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Background: The ability to detect spectro-temporal modulation (STM) cues is closely linked to improved speech-in-noise perception, an ability particularly compromised in individuals with sensorineural hearing loss (SNHL). Impaired STM sensitivity has often been attributed to broadened cochlear tuning and is increasingly being considered a potential early indicator of SNHL.

A new clinical measure, the Audible Contrast Threshold (ACT), adaptively manipulates modulation depth to quantify STM sensitivity. This test has been validated across both normal-hearing and impaired listeners, showing promise as a practical and, importantly, language-independent tool for assessing auditory function. By avoiding linguistic confounds, ACT allows assessment in diverse populations and may support clinical decision-making regarding amplification strategies.

While behavioral studies highlight the real-world relevance of STM sensitivity, less is known about its neural correlates. Cortical responses to acoustic changes, measured as the Acoustic Change Complex (ACC), provide a physiologically grounded metric related to intelligibility. Using electroencephalography (EEG), we seek to characterize how cortical dynamics track STM changes and whether these signatures align with psychophysical markers such as ACT.

Methods: Human EEG recordings were collected while participants listened to STM blocks embedded in broadband noise. Stimulus design alternated between spectrally structured and unstructured sequences, with energy carefully equated across conditions. EEG was acquired using a 32-channel BioSemi system. Post-change neural features, including auditory steady-state responses (ASSR), band-limited power, root-mean-square amplitude, and global field power were analyzed. Logistic regression with stratified five-fold cross-validation was applied to decode modulation depth and classify clean versus noisy conditions. ACT scores were also obtained for all participants, serving as a complementary behavioral reference.

Results: Spectral decomposition revealed that the neural representation of STM stimuli varied across frequency bands. In particular, delta and theta phase coherence following stimulus change increased systematically with modulation depth, consistent with theories of neural entrainment in speech tracking. However, classification accuracy for modulation depth was marginally above chance, suggesting subtle cortical distinctions. In contrast, clean versus noise-added conditions were robustly classified, with area under the ROC curve well above chance, largely driven by ASSR contributions, suggesting that noise-related envelope coding elicits stronger cortical signatures than modulation depth encoding.

Conclusions: Our findings suggest distinct cortical signatures of STM encoding in the human brain, aligning neural entrainment with behavioral measures of modulation sensitivity. By combining EEG with ACT, this work bridges psychophysical and neural levels of analysis, offering a stronger framework for assessing auditory deficits in SNHL. Parallel EEG studies are ongoing using several pre-clinical chinchilla models of SNHL to garner more direct mechanistic insight. Overall, these cross-species insights further support the translational use of STM measures for precision clinical diagnostics and intervention planning.

SU76. External Validation of an Acoustic Change Complex Prediction Model for Speech Perception in Adults With Sensorineural Hearing Loss

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Background: The acoustic change complex (ACC) is a cortical neural response to changes in an ongoing sound, recorded via electroencephalography. Previous work by our group (Vonck et al., 2022) presented an ACC-based prediction model to predict the behavioral speech reception threshold (SRT), which explained 87% of the variance in SRT in normal-hearing and hearing-impaired individuals. This model suggests that the ACC could serve as an objective tool for assessing auditory performance, especially in populations with limited language or cognitive abilities. Our research aims to externally validate the ACC-based prediction model in a broader population with varying degrees of sensorineural hearing loss (SNHL).

Methods: Eighty adults aged 18-65 years (35 men, 45 women) with varying degrees of SNHL participated in this study. ACCs were evoked using acoustic change stimuli consisting of a 3000 ms reference tone at 1000, 2000, or 4000 Hz, gliding to a 300 ms target tone with a 12% higher frequency (i.e. 1000-1120 Hz, 2000-2240 Hz, 4000-4480 Hz). Each participant underwent pure-tone audiometry to determine normal hearing or the degree of SNHL. The SRT was obtained

using the Leuven Intelligibility Sentences Test (LIST). To assess test-retest reliability of the ACC, 52 participants completed a second ACC recording 1-4 weeks after the initial session.

Our aim is to validate the ACC prediction model by Vonck et al. (2022): $SRT = -6.4 + 0.071 \cdot HL + 0.083 \cdot (ACC_{latency} - 100)$, with HL being the pure-tone average at 500, 1000, 2000, and 4000 Hz and ACClatency the average latency of the three ACC recordings.

Results: While the Spearman correlation between predicted and behavioral SRT was strong ($\rho = 0.750$, $p < 0.001$), Bland-Altman analysis revealed poor agreement with increasing discrepancies at higher mean values. Intraclass correlation further confirmed limited reliability of the model across the full range of hearing loss ($ICC = 0.266$, $p = 0.004$). However, ACC test-retest reliability was excellent ($\rho = 0.834$, $ICC = 0.952$, both $p < 0.001$).

Conclusions: External validation revealed that the current ACC prediction model performs adequately only in individuals with normal hearing to mild hearing loss. To generalize across broader SNHL profiles, model refinement is necessary. Further refinement of the model is currently ongoing to improve its applicability. Nonetheless, the high test-retest reliability of the ACC supports its potential as a robust objective measure.

SU77. Estimating Speech Recognition Reference Curves Using Audiometric Measurements

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Background: In clinical practice, audiometric tests—including tone and speech audiometry—are essential for assessing hearing acuity. Tone audiometry measures the softest perceptible sounds per frequency, establishing individual hearing thresholds, while speech audiometry evaluates word recognition across sound levels, producing a speech recognition curve. Comparing an individual's curve to a normal-hearing reference provides insight into speech intelligibility loss and helps optimize hearing aid settings.

Proper calibration is critical for reliable measurements. ANSI and ISO standards define procedures and allowable deviations, enabling consistency across equipment and locations. However, unlike pure-tone audiometry, broadband speech calibration lacks standardized methods. Previous studies have explored different procedures, but changing audiometers or transducers can still affect speech recognition outcomes, even with standard calibration. For example, improved intelligibility of 4.6 dB was reported for Sennheiser HDA200 headphones compared to free-field expectations.

Differences between free-field and headphone listening arise from anatomical acoustic effects and technical characteristics of transducers (e.g., Telephonics TDH39 vs. Sennheiser HDA200). Establishing new reference curves requires many participants and is resource-intensive, while multiple curves increase error risk. This study aims to identify and quantify these differences and develop a predictor to estimate reference curves from technical parameters, eliminating the need for repeated subject testing in the future.

Methods: Thirty-two native Dutch-speaking adults (18–25 years) with normal hearing participated. Eligibility was confirmed via history taking, pure-tone audiometry, bone conduction thresholds, and tympanometry. Audiometric testing was conducted in a sound-insulated room

using an Otometrics Astera2 audiometer under five conditions: free-field (Yamaha MP5S loudspeaker), DD450 headphones, and TDH39 headphones, both with and without audiometer-applied frequency filtering. Calibration followed ISO and IEC standards, and inverse filtering was applied to create unfiltered test material. Speech stimuli consisted of 30 Dutch word lists (Bosman and Smoorenburg, 1995). Speech recognition was assessed monaurally in the better ear, starting at 30–40 dB SPL and decreasing in 5 dB steps over six lists per condition. SRTs and slopes were derived by fitting a Gaussian function using maximum likelihood optimization.

Results: Despite careful calibration and comparable pure-tone thresholds, the DD450 consistently yielded higher speech recognition scores than the TDH39. These differences persisted across filtered and unfiltered conditions, indicating that headphone choice significantly influences speech intelligibility. Although variations in the headphones' frequency response may partly account for these discrepancies, the response characteristics alone do not fully explain the observed effects. Revisions to the calibration procedures in current standards may therefore be recommended to ensure that speech recognition results are independent of the transducer used.

Conclusions: Even when calibration standards are met and hearing thresholds are equivalent, headphone selection can alter measured speech recognition. These findings highlight the necessity of transducer-specific reference curves and motivate the development of predictive models for speech recognition thresholds, enabling reliable clinical assessments across varying audiometric setups.

SU78. The Effect of Spectral Degradation, Level, and Noise on Discourse Comprehension and Listening Effort

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Background: Speech is often masked or distorted by background noise, with communication in background noise often occurring at increased sound levels. Speech perception often decreases at these higher levels, in a phenomenon known as “Rollover.” Hearing loss increases communication difficulty through decreased recognition of single words to impacts of comprehension of longer passages of discourse. One of the best available treatments for moderate-to-profound hearing loss is the cochlear implant (CI), but CI users struggle to communicate in noisy environments. Vcoded speech is used to simulate reduced spectral resolutions experienced by CI users. We investigated communication difficulties in noise under conditions that typically produce rollover, then measured discourse comprehension and listening effort in young normal-hearing adults when they listened to clear unprocessed or vocoded speech under quiet or background noisy conditions at different stimulus levels. We hypothesized that behavioral performance will be maximum in unprocessed speech but will significantly decline at 85 dB SPL in unprocessed and vocoded noisy speech, with this decline followed by increased listening effort at higher sound levels under noisy conditions.

Methods: Nine young normal-hearing adults between the ages of 18-30 years with no neurological or visual impairments listened to 1-1.5-minute-long literary passages, either unprocessed or vocoded. Passages were presented over headphones at sound levels of 35, 55, 65, 75, and 85 dB SPL, in quiet and in background noise at a +10-dB SNR. While listening,

participants focused on a red cross displayed at the center of a screen so an eye tracker could record their pupil dilation as a measure of listening effort. After each passage, participants answered comprehension questions to assess how well they understood the material.

Results: Behavioral performance decreased at the highest sound levels (85 dB SPL) for both unprocessed and vocoded speech in noise, which is consistent with the presence of rollover. Pupillometry revealed that listening effort peaked during unprocessed speech at 75 dB SPL in noise, suggesting that even with non-vocoded acoustic signals, increased intensity in noisy conditions forced subjects to exert more effort while listening to passages. For vocoded speech in noise, pupil dilation was lower than that of unprocessed speech at 75 dB SPL and steadily declined, possibly indicating participants' disengagement with increased task difficulty.

Conclusions: Findings suggest rollover is present during speech in noise but is not correlated with increased listening effort. In young normal-hearing adults, listening effort remained consistent across unprocessed and degraded speech, with no significant relationship with rollover as performance decreased. Additionally, listening effort peaked at a lower level than behavioral performance, indicating that listeners can compensate for a degraded, louder signal with increased effort to maintain behavioral performance. For future research applications, we would maintain challenging auditory conditions while exploring how listening effort is impacted by time-of-day effects.

SU79. Tuning In: Effects of Low-Frequency Allocation and Fine-Structure Timing on Pitch Perception in Cochlear Implant Users

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Background: Conventional cochlear implant (CI) coding strategies, such as the Advanced Combination Encoder (ACE), primarily transmit slow-varying temporal envelope cues while discarding acoustic temporal fine structure (TFS). Although effective for speech in quiet, this limits access to pitch-relevant cues—especially in the low-frequency range crucial for music perception and tonal language comprehension. This study examines whether enhancing low-frequency access and/or reintroducing TFS timing can improve pitch sensitivity in CI users. Using a within-subject psychophysical design, we compare pitch perception across four coding strategies that systematically vary low-frequency allocation and TFS encoding.

Methods: Adult CI users (data collection ongoing) are tested using the Cochlear NIC research interface, which bypasses clinical processors. Four coding strategies are evaluated: (1) ACE-NMT: Baseline ACE implementation from Cochlear's Nucleus MATLAB Toolbox, (2) ACE-LF: Custom ACE variant with expanded low-frequency channel allocation to improve access to fundamental frequency (F0) cues,

(3) HD-ACE: High-definition ACE variant (similar to HDCIS) with increased pulse density to support TFS encoding, and (4) Peak-Derived Timing (PDT): Novel strategy that introduces low-frequency-biased timing by placing pulses at waveform peaks.

Results: All strategies are loudness-balanced, with consistent mapping parameters where possible. Each participant completes three psychophysical protocols, presented in randomized order: (1) Electrode Psychophysics: Discrimination of place, rate, and combined cues to assess

baseline pitch sensitivity, (2) Pure-Tone Pitch Perception: Frequency-difference limens (FDLs) at 110, 220, and 440 Hz, and (3) Musical Pitch Perception: F0-difference limens (F0DLs) for piano tones matched in fundamental frequency.

Stimuli are processed through each coding strategy. Thresholds are estimated using adaptive procedures targeting 75% correct. Linear mixed-effects models (with subject as a random factor) evaluate strategy effects. Secondary analyses assess whether individual baseline sensitivity predicts benefit from enhanced coding.

Conclusions: We hypothesize that ACE-LF will improve FDLs at low frequencies (110–220 Hz) relative to ACE-NMT; HD-ACE and PDT will yield additional improvements by reintroducing TFS timing, especially at lower F0s; and that individual benefits will correlate with sensitivity to place and/or rate cues.

This study provides a systematic, psychophysically grounded comparison of conventional and novel CI coding strategies using both synthetic and ecologically valid stimuli. Findings will guide future CI sound coding aimed at enhancing pitch perception in music and complex listening environments. Preliminary results will be presented.

SU80. The Elusive Bilingual Inhibitory Control Advantage and Its Role in Suprathreshold Hearing: An Investigation

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Background: As multilingual populations grow, it is important to understand how bilingualism influences systems that support speech perception in noise (SPIN). Selective attention and working memory are two cognitive processes that are crucial for filtering task-irrelevant sounds in noisy environments. However, the effect of multiple language exposures on these cognitive processes is still unclear. Here, we hypothesized that bilinguals would outperform monolinguals on auditory tasks that require distraction suppression but show comparable performance on tasks with minimal cognitive demands.

Methods: We recruited 153 participants (ages 18-40) through the online platform Prolific - 65 individuals whose first language (L1) is English and 88 others who are bilingual in English (L1) and Spanish (L2). All participants were right-handed, living in the U.S. or Canada, and had normal hearing. They completed four tasks designed to measure different cognitive abilities related to speech perception and working memory. The matrix sentences task evaluated informational masking by testing participants' ability to filter through multiple speakers while focusing on a target speaker. The auditory Stroop task assessed distraction suppression by having participants respond to prompts while ignoring conflicting auditory information. The Modified Rhyme Task assessed energetic masking by having participants identify a target word masked by inharmonic tones. The n-back test measured working memory by requiring participants to recognize stimuli presented earlier in a sequence.

Results: Bilingual participants showed a decreased Stroop cost, suggesting a bilingual advantage in distraction suppression. However, no group differences were observed in working memory, or the informational masking task. Surprisingly, monolinguals outperformed bilinguals in the energetic masking task, indicating that bilingualism may come with a greater cost of lexical recovery when retrieving words presented without linguistic context. Overall, findings support a conditional bilingual advantage confined to tasks with inhibitory control demands.

Conclusions: Bilingual listeners showed enhanced distraction suppression and inhibitory control. Ongoing analyses are focused on examining duration and degree of bilingualism in explaining variance on SPIN performance. Future work will test additional language pairings, broader age ranges, and real-world outcomes (e.g., listening effort, fatigue, device use) to identify when bilingual experience confers benefit, or cost, for everyday listening. Clinically, this condition-specific bilingual advantage has implications for personalized counseling and diagnostic testing.

SU81. Differential Effects of Masker Type on Speech-In-Noise Perception in ADHD

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Background: Attention Deficit Hyperactivity Disorder (ADHD) is associated with impairments in working memory and cognitive control, which are critical for goal-directed sensory behavior. These deficits may make it difficult for individuals with ADHD to selectively attend to speech in complex acoustic environments. Prior work suggests adults with ADHD experience increased cognitive load and expend more effort than neurotypical peers during speech perception-in-noise (SPIN) tasks. This effort is likely tied to limitations in working memory and attentional control, which are necessary for separating target speech from competing sounds. We hypothesize that individuals with ADHD will show lower accuracy on a SPIN task with informational masking compared to controls. We further expect that working memory and distraction suppression ability will predict SPIN task performance.

Methods: This study compared SPIN processing in individuals with and without ADHD in energetic and informational masking conditions. The Modified Rhyme Test with an inharmonic tone complex served as an energetic masker, while the Matrix Sentences task was paired with a non-overlapping informational masker to probe auditory processing at higher levels. The signal-to-noise ratio (SNR) between target and masker was varied to determine thresholds. Working memory was assessed via an n-back task ($n = 1-3$). Distraction suppression was measured using an Auditory Stroop task, calculating Stroop cost as the difference in reaction times between incongruent and congruent trials.

Results: Participants with ADHD demonstrated significantly higher SNR thresholds on the informational masking task relative to neurotypical peers, but no group difference emerged for the energetic masking condition. Initial group comparisons of distraction suppression via Stroop cost were not significant; however, after controlling for age, gender, and baseline reaction time, the ADHD group performed significantly worse. No group differences were found in working memory as measured by the n-back task.

Conclusions: The observed SPIN deficit under informational, but not energetic, masking indicates that ADHD-related deficits in speech perception occur beyond the cochlear level. The significant Stroop cost difference supports the role of reduced distraction suppression in explaining poorer SPIN outcomes for ADHD individuals. These results suggest attentional control, rather than initial auditory encoding, underlies the group differences in SPIN performance. Ongoing analyses will include questionnaire data on self-reported ADHD symptoms and medication use, allowing exploration of relationships between symptom severity, medication, and SPIN perception.

SU82. Cross-Species Physiological Measures of Spectrotemporal Modulation Processing

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Background: Restoration of speech audibility through amplification is a cornerstone of current audiological practice. Yet, individuals with sensorineural hearing loss (SNHL) often struggle to understand speech, even when it is audible, particularly in noisy environments. Psychophysical measures of sensitivity to spectro-temporal modulations (STM) have emerged as language-independent assays of suprathreshold auditory fidelity that can reliably predict speech-in-noise performance. However, behavioral testing is impractical in infants and other difficult-to-test groups and the neural correlates of this predictive power remain unknown. The acoustic change complex (ACC), an EEG response to modulation onset, may provide an objective neural correlate of STM sensitivity. This project seeks to establish normative baselines and optimized testing parameters for the STM-based ACC by collecting data in both normal-hearing humans and chinchillas. These foundational measures will then provide the framework for subsequent application across the continuum of normal hearing and SNHL in both human listeners and preclinical chinchilla models.

Methods: Human participants undergo otoscopy and full audiometric evaluation to confirm normal hearing. Behavioral measures include word identification in noise using the Modified Rhyme Test (MRT) materials, and STM sensitivity for noise carriers as in Zaar et al., (2023; 2024). Electroencephalography-based (EEG-based) ACCs were acquired as neural metrics of STM processing. Stimuli for ACC measurements were 2 seconds long consisting of a 1-s unmodulated reference segment smoothly transitioning to a 1-s spectrotemporally modulated segment. Responses to both tonal and noise carriers were collected. ACC responses are analyzed for reference-to-STM transition as a function of modulation depth and will be compared directly to behavioral STM thresholds in human listeners. Analogous stimuli are also employed for chinchillas with ACC responses measured using both subdermal electrodes and a 32-channel mini-EEG cap. Specifically, because peripheral frequency tuning in chinchillas is known to be substantially broader than psychophysically measured frequency selectivity in human listeners (Temchin et al., 2008; Shera et al., 2010), measurements in chinchillas span a wider range of STM parameters.

Results: Preliminary data show that ACC responses are measurable in both species. In human listeners, preliminary data also show that behavioral STM thresholds are predictive of speech-in-noise outcomes, replicating the work by Zaar et al. (2023; 2024)

Conclusions: These results highlight the feasibility of using the ACC as a candidate neural marker of suprathreshold auditory processing relevant for speech-in-perception. Data collection in both species is ongoing with the goal of expanding the sample. Human ACC measures will be compared to behavioral STM measurement to evaluate the translational potential of this approach to populations and settings where behavioral testing is not feasible. Chinchilla ACC norms established through this work will be used in future studies in SNHL chinchilla models.

SU83. The Long-Term Impact of Childhood Mild Traumatic Brain Injuries on Adult Auditory Processing Abilities

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Background: Mild traumatic brain injury (mTBI), or concussion, is highly prevalent in children yet long-term neural effects are still unclear. While many children appear to recover, prior studies suggest lasting deficits in auditory working memory, speech-in-noise perception, and increased listening effort that can persist into adulthood. Normal aging independently contributes to similar declines in auditory processing abilities causing everyday communication challenges. However, the interaction between early-life concussion and age-related auditory decline remains largely unexplored, leaving a critical gap in our understanding of long-term outcomes due to mTBI across the lifespan.

Methods: 252 North American English-speaking adults, aged 18-72, were recruited via an online platform, Prolific. All participants reported normal hearing, and passed a preliminary headphone check and hearing screening. A total of 104 participants self-reported a history of childhood mTBI, while 148 served as age- and sex-matched controls. Participants completed a concussion history questionnaire, post-concussion symptom survey, and the Hearing Handicap Inventory for Adults (HHIA). Auditory assessments included speech-in-noise perception (Matrix Sentences), auditory working memory (N-back), selective attention and inhibitory control (Auditory Stroop), and energetic masking (Modified Rhyme Test).

Results: Adults with childhood mTBI performed significantly worse in a speech-in-noise task emphasizing informational masking, while no differences were observed in energetic masking conditions. Additionally, a childhood history of mTBI was also significantly associated with decreases in auditory working memory assessed using an n-back task, and inhibitory control assessed using Auditory Stroop. While aging and mTBI each negatively impacted performance on the inhibitory control and the informational masking task, mTBI did not amplify age-related declines across the lifespan, suggesting the effects of concussion are long-lasting but largely independent of aging.

Conclusions: Childhood mTBI has persistent negative effects on auditory processing in adulthood, particularly in tasks requiring working memory, inhibitory control, and involving informational masking. These deficits appear independent of aging and separate from energetic masking, underscoring the long-term impact of pediatric concussion. Clinically, results emphasize the importance of considering concussion history when evaluating adults with speech-in-noise complaints despite normal audiometric thresholds, as these patients may present with central rather than peripheral deficits. Ongoing analyses will examine the role of injury

frequency, age of occurrence, and subjective hearing handicap in shaping outcomes. Together, this work highlights the need for proactive monitoring, patient counseling, and development of rehabilitation strategies to reduce the enduring consequences of childhood mTBI.

SU84. Using Mobile EEG for Tracking Auditory Attention in Realistic Scenarios: Introducing Attention Switches and Conversations

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Background: Auditory attention decoding (AAD) identifies which sound source a listener focuses on in multi-talker settings. This is key for future hearing aids (HAs), where neural signals could guide selective amplification of attended speech. Generally, there has been a heavy focus within AAD to use controlled lab setups with clean, audio-only stimuli and record brain activity via stationary equipment such as magnetoencephalography (MEG) or wired electroencephalography (EEG). While effective, these lack ecological validity, as real-world listening involves attention switching, visual cues, and eye gaze changes.

Recent work moves toward realistic scenarios with more complex tasks (e.g., switching attention, audiovisual stimuli), and mobile devices such as ear-centered EEG arrays (cEEGrids) and wireless EEG caps are important steps toward future brain-controlled HAs. Here, we recorded mobile EEG from 24 normal-hearing participants in a novel audiovisual (AV) multi-talker paradigm with three AV conditions: sustained attention, switching attention, and attending to a podcast conversation.

Methods: The participant sat in a sound-attenuated room with three monitors at azimuths 0 degrees and 30 degrees left/right, and four loudspeakers at 30 and 90 degrees left/right. Two AV stimulus sets were used: (1) single talkers speaking to the camera and (2) a two-person podcast conversation. Conditions included sustained attention, switching between talkers, and attending either the AV conversation or a side audio-only speaker.

EEG was recorded with a 44-channel scalp cap and 20-channel cEEGrids, eye movements with a static tracker, and sound with two ceiling microphones. Linear temporal response functions (TRFs) were applied forward (predicting EEG from speech) and backward (reconstructing speech from EEG). Analyses included TRF peaks (P1, N1, P2), reconstruction correlations, optimal lags, and classification, for comparison between speech features, attention, EEG setups, audio sources, and conversation settings.

Results: Scalp EEG revealed significant attention-related P2 peak differences between attended and ignored speech. cEEGrid data showed weaker correlations, highlighting the need to investigate optimal preprocessing settings further. Performance did not differ between sustained and switching conditions, suggesting robustness to attention shifts. Classification accuracy ranged from 55–70% across decision windows (1.1–35 s), supporting feasibility in varied listening tasks with mobile EEG.

Conclusions: This study extends mobile EEG beyond single-talker audio-only settings to AV sustained attention, switching, and conversations. While cEEGrids showed weaker attention modulation, scalp EEG provided robust results with clear TRF signatures and stable model performance. Backward models generalized across tasks, with classifiers exceeding chance in all conditions. These findings bring neural tracking closer to realistic applications and future brain-controlled HAs.

SU85. Speech Perception in Quiet and Noisy Environments in Mandarin-Speaking Cochlear Implant Users With Low Frequency Residual Hearing

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Background: The residual hearing (RH) is important for the rehabilitation results after cochlear implant (CI) surgery.

Methods: Thirty-seven sensorineural hearing loss (SNHL) patients (41 ears) with different degree of low frequency residual hearing (LFRH) underwent CI at our department from June 2017 to July 2024 were enrolled. Speech recognition included the monosyllable and disyllabic word in quiet, the sentence in quiet, the consonant, vowel, and lexical tone in quiet, and sentence in noisy environment. Correlations between preoperative LFRH and improvement in postoperative speech recognition rate after surgery were analyzed.

Results: Patients enrolled included six ears implanted with SY28, five with F28, six with MS, two with 422, 10 ears 512, two with 622, five with CA, two with SJ, and two with 522 electrodes. The mean age at CI surgery was 18.9 years (range: 4-44 years). The mean threshold of 125, 250, and 500Hz was 52.0dB (5-80dB), 59.9dB (35-90dB), and 73.7dB (30-105dB) HL, respectively. The preoperative recognition rate was 16.5% in monosyllable, 18.0% in disyllabic, 27.8% in sentence in quiet. The postoperative (mean, 7.6 months) rates were 65.8% in monosyllable, 73.0% in disyllabic word, 85.5% in sentence in quiet, 64.8% in consonant, 67.3% in vowel, 84.0% in lexical tone, and 4.1dB signal noise rate (SNR) in sentence in noise. The speech recognition was significantly improved at nearly one year after CI surgery ($p < 0.05$). The preoperative threshold of 125 Hz was significantly correlated with the improvement of disyllabic word recognition post CI ($p=0.0382$, $r=0.1056$).

Conclusions: Patients with residual hearing experience a significant improvement in speech recognition approximately one year after CI, and the preoperative 125Hz threshold is correlated with the postoperative improvement in disyllabic word recognition.

SU86. Frontal Cortex Modulation of Frequency Tuning During Word Recognition in Noise in Middle-Age and Older Adults

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Background: Difficulties in understanding speech in noisy environments present a significant challenge for many older adults. Pre-stimulus frontal activity appears to support word recognition in noise, including for older adults with hearing losses. One potential mechanism for

this performance benefit is frontal modulation of auditory cortex, a hypothesis supported by evidence that inhibition of cingulate cortex diminishes auditory discrimination in animals. We tested the hypothesis that pre-stimulus frontal cortex activity modulates or alters frequency tuning in auditory cortex during a word recognition in noise task and examined the extent to which tuning effects interact with age-related hearing loss.

Methods: A retrospective analysis was performed with data from 45 middle-aged and older participants (average age = 64.7 ± 7.3 years) who had normal hearing to moderate hearing loss. Participants were asked to identify words presented in speech-shaped noise (speech: 88 dB SPL, noise: 73 dB SPL; +15 dB signal-to-noise ratio). The words and noise were presented in the relative quiet between sparsely collected functional magnetic resonance imaging (fMRI) data. Spectral power in one-third-octave bands was computed for each word. For each band, a GLM was fitted that modelled words on trials with higher vs. lower pre-stimulus frontal-cortex activity, as well as a parameter for spectral power in that band. Voxel-wise characteristic frequency (CF) in auditory cortex was then defined by the band with the strongest BOLD association. Frequency tuning was examined based on the strength of BOLD associations with spectral power for CF and non-CF bands. The strength of these associations was compared between high- and low-frontal activity trials.

Results: Elevated pre-stimulus frontal cortex activity was significantly associated with higher auditory cortex BOLD contrast for both CFs and non-CFs, across subjects and speech frequencies [paired $t(224) = 3.13$, $p = 0.002$]. Participants with more hearing loss also appeared to show smaller auditory cortex responses to spectral power for specific one-third octave bands. For example, elevated 0.5 kHz pure-tone thresholds were associated with significantly smaller increases in CF effects for trials that were preceded by high pre-stimulus frontal activity [$r(31) = -0.39$, $p = 0.02$].

Conclusions: The results suggest that frontal cortex modulates auditory cortex frequency tuning during word recognition in noise by increasing the responsiveness of auditory cortex to variation in spectral power, but to a lesser extent of listeners with more hearing loss. Together, these findings are consistent with frontal modulation of auditory cortex during word recognition in noisy conditions.

SU87. Analyzing Deep Learning and Classical Machine Learning Methods for Classifying Communication and Head Movement Behaviors in Live Multi-Participant Conversations

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Background: Head movements play a pivotal role while engaged in multi-talker conversation by providing non-verbal feedback to partners and enhancing a listener's ability to separate sound sources. Commercial hearing-aids with an on-board IMU (Inertial Measurement Unit, i.e., accelerometers) typically use that information for step-counting and activity levels. At least one device uses it as an input to their environment classifier and integrated directional microphone. None, however, use the IMU to detect specific patterns of head movements to predict behaviors such as nodding, head shaking, listening to a person versus a video, or talking. Training an

automatic classifier to accurately detect these behaviors first requires collecting head movement data during multi-person conversation and laboriously annotating each behavior-type for each participant with high temporal precision. From that point, with the goal of training the most accurate model and integrating with the hardware in the hearing aid to improve device performance, the question is how best to model the data. To address this gap, we collected accelerometer data during natural multi-person conversations and paired it with detailed human annotations of communication and head-movement behaviors.

Methods: Head-movement data was collected from three cohorts of young, normal-hearing individuals (three per cohort) in a controlled, conference-room setting during 50-minute multi-talker conversations. Participants wore hearing aids with on-board accelerometers, and audio-video was recorded for each talker. Videos were manually annotated for communication and head-movement behaviors, including conversational turns and nonverbal cues such as tilts and nods. Temporal and spectral features were extracted from the accelerometer data (windowed into 1-second segments) corresponding to roll and pitch movements. These features, combined with the annotated behaviors, were used to train and test machine learning models. Models were trained on data from all but one participant and then tested on the held-out participant, repeating this procedure across all individuals.

Results: Several deep learning and classical machine learning models were compared for classifying communication behaviors (e.g., talking, listening, watching video) and head orientations (e.g., turning left or right, facing down, facing forward). More specifically, various sequence-to-sequence models, a state-of-the-art deep learning technique, were utilized. These models incorporated modern architectural components such as transformer networks. Multiple performance metrics were used to evaluate models, and results suggest that modern deep learning models outperform classical machine learning methods by significant margins. Classification performance improved further when temporal sequence information was incorporated.

Conclusions: These results indicate that during multi-talker conversation, hearing-aid accelerometers can automatically classify stereotypical behaviors with high temporal resolution (1-second). Even when tested on unseen subjects, the models remained reliable. This establishes a foundation for more advanced approaches that combine behavioral and movement patterns, further integrate temporal dynamics, and incorporate additional inputs to improve accuracy and ecological validity.

SU88. Cortical Response Differences in Hearing-Impaired and Normal-Hearing Older Adults During Speech Perception in Noise

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Background: At the neural level, auditory streams develop along a hierarchical pathway: primitive acoustic features are encoded as early as the auditory brainstem and continually refined up to the primary auditory cortex; from there, perceptual-feature representation and ultimately object and category-specific representations are formed along the ventral cortical pathway. To investigate how selective attention operates within this hierarchical pathway, we conducted an experiment using electroencephalography (EEG). Our previous studies with younger and older

listeners have shown prominent late-latency cortical responses to changes in spatial location for steady-state noise and speech targets. The present experiment specifically investigated how hearing impairment in older listeners affects these late-latency cortical responses.

Methods: Older, hearing-impaired adults ($n = 20$) and older, normal-hearing adults ($n = 18$) were tested in the free field. Listeners were presented with a speech stream either in quiet or in background babble at 6-dB signal-to-noise ratio (SNR). Speech materials were taken from a corpus of ~2500 monosyllabic words spoken by a male voice with an interstimulus interval of 100 ms, and babble consisted of non-English conversations from 10 talkers. Listeners either passively listened or responded by button press after the speech stream changed location among 5 possible locations in the front hemifield. Cortical responses were measured using a 64-channel EEG system. EEG preprocessing was performed using the Brainstorm analysis software, followed by custom signal processing and statistical analysis in MATLAB.

Results: The EEG global field power (GFP) was characterized by N1, N2, and P3 deflections. Notably, all components were observed in both active and passive tasks, consistent with prior findings in younger normal-hearing listeners. Preliminary results suggest that participants' P3 responses to speech occurred earlier in passive conditions compared with active attention ones. During passive listening, P3 activity was higher in normal-hearing subjects than those with hearing impairment; this difference was not observed during the later P3 peaks in the active attention tasks. Earlier components (N1 and P2) were also significantly larger in normal-hearing individuals, particularly during passive conditions. Additionally, hearing impairment was associated with differences in P3 amplitude across specific spatial angles.

Conclusions: Shorter latencies could reflect primarily bottom-up, stimulus-driven processes, whereas later responses might be associated with top-down, task-related processing. The fact that the prominence of these components diverge across the listening groups could be indicative of poor audibility, as well as possible compensatory effects. Future work may include effects of intervention (e.g., amplification) on emphasizing bottom-up processing effects in the hearing-impaired listener group

SU89. Central Encoding Deficits and Compensatory Behavioral Strategies After Peripheral Deafferentation in Guinea Pigs

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Background: Exposure to moderate-to-intense noise can cause temporary threshold shifts and permanently decreased auditory brainstem response (ABR) wave I amplitudes. These decreases are thought to arise from the peripheral deafferentation (PD) of high-threshold auditory nerve fibers that encode sounds in loud listening conditions. However, the extent to which decreased ABR wave I amplitudes correlate with speech-in-noise perception deficits has been a subject of much debate, with recent studies yielding conflicting results. Resolving this question is critical for understanding the neural underpinnings of 'hidden' hearing loss and developing effective diagnostic and therapeutic strategies.

Methods: To help resolve this question, we developed a guinea pig model to investigate PD effects on a complex sound recognition task (vocalization-in-noise categorization). We induced

PD with noise exposure and confirmed that ABR wave I amplitudes were permanently decreased while thresholds recovered to ~5 dB of baseline.

Results: We found that local field potentials (LFPs) in the auditory cortex were altered after PD, indicating changes in central sound encoding. We expected this combination of peripheral and central deficits to significantly affect categorization behavior. Surprisingly, however, animals continued to perform well in the categorization task. Behavioral analyses revealed that the animals adopted different compensatory strategies, with a high degree of individual variability, to overcome the underlying encoding deficits. To determine whether the high performance after PD could be the result of engaging top-down mechanisms, we used pupillometry in passive listening animals to measure behavioral correlates of largely bottom-up processing. We found that vocalization-in-noise categorization thresholds were indeed affected at loud sound levels, consistent with high-threshold fiber loss.

Conclusions: These preliminary results suggest that top-down mechanisms can powerfully mask PD-induced deficits present in the ascending auditory pathway. We propose that taking these central compensatory mechanisms into account is essential for resolving the debate on the relationship between PD and speech perception deficits.

SU90. Dose-Dependent Ototoxicity Effects of Gentamicin and Ethacrynic Acid in a Chinchilla Model of Snhl

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Background: In preclinical models, sensorineural hearing loss can be induced by targeting specific cochlear structures using controlled noise exposure or ototoxic drugs. In chinchillas, carboplatin exposure selectively targets inner hair cells. A mild noise exposure protocol causes reversible threshold elevations but permanent synaptic loss between auditory- nerve fibers and IHCs (cochlear synaptopathy), whereas a moderate noise exposure protocol produces permanent damage to IHCs, outer hair cells, and synapses. Additionally, previous studies have shown that high combined but single doses of ethacrynic acid and gentamicin lead to near-complete OHC destruction, though these reports have focused primarily on anatomical and histological outcomes. The goal of this research is to develop a dosing strategy that induces gentamicin-related OHC-based hearing loss with threshold shifts like those produced by the moderate noise exposure protocol, while preserving IHCs, and to investigate the associated physiological changes in cochlear function. Optimizing efficient gentamicin protocol will provide a reliable model for studying OHC-specific contributions to SNHL and for evaluating therapeutic strategies.

Methods: Two approaches were used to induce OHC damage in chinchillas. In the first, gentamicin was administered intramuscularly in combination with intravenous ethacrynic acid, which has been used in the past to achieve OHC damage with single doses. In the second, animals received only repeated systemic gentamicin dosing over several. Hearing function was evaluated at baseline and at multiple post-treatment time points using auditory brainstem responses, middle ear muscle reflexes, and otoacoustic emissions, including distortion-product, stimulus-frequency, and transient-evoked measurements.

Results: Gentamicin combined with ethacrynic acid produced dose-dependent effects on cochlear function in chinchillas. While lower doses caused only subtle increases in ABR thresholds and small reductions in OAEs, higher doses resulted in moderate-severe to severe threshold shifts and near-complete loss of OAEs, consistent with at least widespread OHC destruction. However, the extent of this damage exceeded what typically observed in the moderate noise exposure protocol, which produce more moderate threshold elevations and preserve some OAEs, suggesting only partial OHC dysfunction. The higher gentamicin dose with ethacrynic acid was also associated with systemic health complications. Together, these findings suggest that although gentamicin with ethacrynic acid reliably induces OHC-related hearing loss, its severity can surpass the moderate noise exposure model and highlights the need to optimize dosing to achieve moderate threshold shifts while preserving IHCs, which we are continuing to pursue.

Conclusions: These preliminary findings underscore the importance of carefully optimizing gentamicin–ethacrynic acid dosing strategies to selectively damage OHCs without affecting IHCs. Establishing such a protocol will provide a consistent and efficient experimental model of OHC-specific SNHL. Beyond its research value, this model will contribute to the development of more precise diagnostic approaches that can eventually be translated to clinical practice, improving the identification of cochlear pathologies, and guiding more effective therapeutic interventions.

SU91. Relationship Between Distorted Tonotopic Coding and Suprathreshold Spectrotemporal Modulation Perception in Sensorineural Hearing Loss

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Background: Threshold audiometry and restoration of speech audibility through amplification are cornerstones of current audiological practice. Yet, individuals with sensorineural hearing loss (SNHL) often struggle to understand speech, even when it is audible, particularly in noisy environments. Psychophysical measures of sensitivity to spectro-temporal modulations (STM) have emerged as language-independent assays of suprathreshold auditory fidelity that can reliably predict speech-in-noise performance. However, the mechanistic basis underlying individual differences in STM sensitivity among patients with SNHL remains unknown. Emerging cross-species evidence from our team and others suggests that SNHL not only broadens auditory filter "tips," as traditionally recognized, but also distorts the fundamental tonotopic organization of the cochlea. Specifically, temporal responses of the cochlear base can be commandeered by "off-frequency" (lower frequency) sound fluctuations. Furthermore, chinchilla models reveal that the degree of distorted tonotopy varies according to the specific cochlear pathology underlying SNHL. For instance, suprathreshold tonotopy remains largely intact in metabolic hearing loss but is significantly distorted in noise-induced hearing loss, which is associated with outer hair cell damage. Consistent with animal models, human studies demonstrate that individuals with similar clinical hearing profiles can exhibit widely varying tuning-curve tip-to-tail ratios (TTRs) and associated degrees of distorted tonotopy. These individual variations in distorted tonotopy are a candidate mechanism to explain why patients with similar audiograms can differ markedly in their STM sensitivity and speech-in-noise

comprehension. This study directly tests whether individual variations in markers of distorted tonotopy predict individual differences in STM sensitivity and speech-in-noise performance.

Methods: To investigate the relationship between distorted tonotopy and STM sensitivity, we recruited individuals along the continuum of normal hearing to symmetric moderate SNHL (N=32 thus far) to participate in lab measurements to gather STM thresholds, speech-in-noise (SPIN) thresholds, and psychophysical tuning curves. Separately, anonymous participants (N=53 thus far) were recruited to participate in online web-based measurements where STM thresholds, SPIN thresholds, and tuning-curve TTRs.

Results: Linear modeling analysis of in-lab data revealed that TTRs, a correlate of distorted tonotopy, are predictive of aided SPIN scores, and online data replicated this association. Additionally, online STM thresholds predict SPIN replicating the results from Zaar et. al (2024). In turn, STM thresholds were predicted by TTRs.

Conclusions: These preliminary data showing associations between TTRs, STM sensitivity and SPIN scores are consistent with the notion that distorted tonotopy is a prominent contributor to suprathreshold hearing outcomes in SNHL. Further data collection is ongoing.

SU92. Fidelity and Validity of the French-Canadian Version of the Communication and Acceptance Scale (FR-CAS)

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Background: Hearing loss can significantly affect various aspects of daily life. Constantly needing to ask others to repeat themselves or having to explain one's hearing difficulties and coping strategies can lead to feelings of fatigue, frustration, or discouragement. Over the years, numerous questionnaires have been validated to assess the functional impacts of hearing loss. However, hearing care professionals in French-speaking regions of Canada have access to very few tools that have been validated in their language. The Communication and Acceptance Scale (Öberg and al., 2020) is a Swedish questionnaire that comprises 22 items, including 18 statements rated on a Likert scale ranging from "totally agree" (1 point) to "totally disagree" (5 points). This study aimed to 1) translate and culturally adapt the Communication and Acceptance Scale (FR-CAS), and 2) evaluate the metrological qualities of its French-Canadian version.

Methods: This study was conducted in two phases: the first involved cross-translation, and the second focused on validation. For the first phase, the questionnaire underwent a rigorous double-translation process, accompanied by consultations with French-speaking and Swedish-speaking individuals with knowledge in the field of communication disorder, as well as audiologists, to ensure linguistic, conceptual and cultural equivalence. For the second phase of the study, a total of 90 participants were recruited. All were aged 18 or older, fluent in French, and self-identified as living with hearing loss. Participants were asked to complete three instruments: the (FR-CAS), the Hearing Handicap Inventory for the Elderly- Screening version (HHIE-S), and the Échelle de mesure de difficultés d'audition (ÉMDA). To assess test-retest reliability, participants completed

the FR-CAS a second time following a two-week interval and intraclass correlation analysis was conducted. A Cronbach alpha was measured to assess internal consistency and Spearman correlations were conducted to assess concurrent validity between the questionnaires.

Results: The internal consistency of the overall scale was strong, with a Cronbach's alpha of 0.81. Test-retest reliability was also robust ($r = 0.810$, $p < 0.001$), indicating good temporal stability. Concurrent validity was moderate, but statistically significant, when comparing total scores from the FR-CAS with both the HHIE-S ($r = -0.682$, $p < 0.001$) and the EMDA ($r = -0.615$, $p < 0.001$). All correlations were similar to those reported for the original Swedish CAS.

Conclusions: The French version of the Communication and Acceptance Scale (FR-CAS) demonstrated strong validity and reliability, establishing it as a new tool for hearing professionals in French-speaking regions of Canada. It offers a meaningful way to assess individuals' acceptance of hearing loss and its effects on communication and emotional well-being.

SU93. Longitudinal Changes in Acoustic Absorbance in Cochlear Implant Recipients

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Background: Patients often experience decrements in acoustic hearing sensitivity following cochlear implantation.

When hearing recovers, conductive hearing loss is suspected (Chole et al., 2014). When hearing loss persists, this is often attributed to cochlear trauma. Some post-surgical changes impacting sound transmission are transient (Racca et al., 2022), though permanent ones have also been suggested (Scheperle and Hajicek, 2020). Cochlear conductive hearing loss may also contribute (Banakis Hartl et al., 2016). Correctly identifying the mechanism(s) responsible for post-operative hearing loss is critical to improving hearing preservation outcomes and to identifying the most appropriate medical and audiological management for the individual patient.

This study focuses on evaluating middle ear sound transmission using longitudinal absorbance measurements in newly implanted CI recipients. The aims include (1) establishing the incidence of suspected conductive hearing loss, (2) describing the timeline and frequency characteristics of recovery, and (3) characterizing permanent changes to middle ear transmission. Individual variability is of interest as changes post implantation may arise from multiple factors impacting different frequency regions.

Methods: Fifteen cochlear implant (CI) recipients have participated to date with various lengths of follow-up post-surgery. Video otoscopy and wideband acoustic immittance measures are being performed serially: at surgery (prior to implantation) and at each post-operative visit (initial activation, 0.5-, 1-, 3-, 6-, and 12-months post activation.) Otoscopic images are evaluated post-hoc for evidence of hemotympanum, effusion, or other notable findings. Baseline absorbance measurements are compared to published normative data, and post-operative absorbance is compared to the subject's own pre-operative baseline.

Results: Pre-operative measurements were not always normal. Some ears had a history of previous ear surgery (e.g. myringotomy tubes) or external ear abnormalities (e.g. exostosis). No ear was excluded; each subject's pre-operative measurements served as their baseline for comparison with their post-operative results

Evidence of hemotympanum/effusion was seen frequently at initial activation (~2 weeks post-surgery). Absorbance for ears with abnormal otoscopic impressions will be compared with absorbance for ears with normal otoscopic impressions. Effusion was visually observed up to 2 months. The frequency patterns, direction, and magnitude of absorbance changes relative to pre-operative baselines were highly variable across subjects. Within a subject, the absorbance systematically changed over time, though resolution to baseline was observed in only a few cases. These patterns will be quantified further as more data are available.

Conclusions: Sound transmission through the outer and middle ear is impacted by cochlear implantation. The effects are observed immediately post-surgery and change over time. Otoscopy can help identify changes to the middle ear system visible through the tympanic membrane, while absorbance patterns provide insight into changes that are less visible and may persist beyond the acute healing phase.

SU94. A Multi-Biomarker Battery for Stratifying Complex Sensorineural Hearing Loss and Predicting Listening Outcomes

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Background: Complex sensorineural hearing loss (SNHL) can involve multiple components of the peripheral and central auditory pathways, but standard clinical measures of hearing like the audiogram are primarily sensitive to OHC dysfunction. Other pathologies, such as cochlear synaptopathy or inner-hair-cell dysfunction, can remain undetected despite their detrimental effects on neural coding of sound and perception. These hidden pathologies, with or without co-occurring OHC dysfunction, are likely to contribute to variability in perception and treatment outcomes. Non-invasive biomarkers that detect the diversity of cochlear pathologies potentially present in SNHL are needed to better understand and address the complaints of individuals with hearing difficulties. Pre-clinical animal models of SNHL provide mechanistic insight into the effects of specific pathology profiles on physiological biomarkers, and studies in humans improve our understanding of the perceptual consequences of hearing loss. In this cross-species study, we use a battery of biomarkers in both human and animal subjects to dissect the clinical category of SNHL, expanding previous work to investigate multiple subtypes of hidden and overt hearing loss.

Methods: Pre-clinical chinchilla models of four types of sensorineural injury were used to assess the sensitivity of each biomarker to isolated and co-occurring pathologies, induced either with noise or ototoxic drugs. Before and after exposure, animals completed a physiological battery including auditory brainstem response (ABR) thresholds, ABR to a high-level click, envelope following response (EFR), otoacoustic emissions (OAEs), and the middle ear muscle reflex (MEMR). Human participants with normal hearing or mild to moderate SNHL were also tested

with the same physiological measures. In addition, human subjects completed three speech-in-noise tasks to relate physiological measures of hearing to speech perception.

Results: In chinchillas, biomarkers showed changes after exposure in at least one group, but no single biomarker was sufficient to accurately classify animals by their exposure group. Classification accuracy significantly increased when a combination of biomarkers was used. The most accurate model included a combination of distortion product OAEs, MEMR strength, phase-locking value, pure-tone average, EFR magnitude, and ABR Wave I amplitudes. In human subjects, including physiological measures improved the identification of individuals with poor speech-in-noise performance. Specifically, MEMR and stimulus frequency OAEs were sufficient for identifying the poorest performing individuals with normal hearing thresholds.

Conclusions: This work highlights the critical need to consider biomarkers beyond the audiogram for diagnosing hearing loss. A combination of measures sensitive to both OHC and non-OHC dysfunctions is needed to improve the diagnostic specificity of SNHL. Additionally, a large, standardized dataset will be necessary for more detailed profiling that takes advantage of advances in machine learning based classification techniques. To effectively analyze the contributions of these factors to hearing impairment, a multi-metric physiological, cross-species profiling approach will be essential.

SU95. Predicting Profiles of Cochlear Pathology From Non-Invasive Physiological Biomarkers

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Background: Temporal-bone histology shows that damage to outer hair cells, inner hair cells, the stria vascularis, and the auditory nerve contribute to sensorineural hearing loss.

Physiological measures such as otoacoustic emissions, the middle ear muscle reflex, and the auditory brainstem response are used both in research and clinically to detect these pathologies and to aid in identifying the site of lesion. However, it remains difficult to establish a one-to-one correlation between physiological responses and histological profiles, especially when more than one pathology is present (as is typically the case with complex sensorineural hearing loss). In this study, we examined the relationship between individual histological profiles of cochlear pathology in several pre-clinical chinchilla models and responses on a battery of physiological tests sensitive to both OHC and non-OHC dysfunctions. Our goal is to identify individual profiles of sensorineural hearing loss through physiological biomarkers of cochlear pathology.

Methods: Chinchillas with and without experimentally induced hearing loss (noise or ototoxic drugs) were used. Distortion product otoacoustic emissions (DPOAEs), stimulus frequency otoacoustic emissions (SFOAEs), wideband middle ear muscle reflex (MEMR), auditory brainstem responses (ABR), and envelope following responses (EFR) to a rectangular amplitude modulated tone were collected on each animal. Pre- and post-treatment data were collected for exposed animals. After all testing was completed, animals were euthanized and perfused. Cochleas were harvested, post-fixed, and decalcified. Cochleas were then bisected through the modiolus, immunolabeled, and dissected into half turns. Specimens were labeled for hair cells (Myo7a), afferent neurons (NFH), hair cell presynaptic ribbons (CtBP2), and cholinergic efferent

neurons (ChAT). In a subset, stereocilia were also labelled (ESPN). Confocal z-stack images were obtained at .5, 1, 2, 4, and 8 kHz cochlear locations.

Results: The noise-exposure protocol resulted in a mild to moderate elevation of ABR thresholds and reduced DPOAE amplitudes. Most of these animals, however, showed intact hair cells at the tested cochlear locations. Gentamicin exposed animals showed profound elevation of ABR thresholds and complete loss of hair cells. Consistent with prior findings, hair-cell counts alone underestimated the effects on auditory physiology. Many animals with overt elevations in hearing sensitivity showed little to no loss of hair cells. Further image analysis will also quantify the contribution of stereocilia dysfunction to responses on physiological assessments in the absence of hair cell loss.

Conclusions: Profiling individual degrees of pathology across multiple components of the cochlea is critical to understanding the contribution of various pathologies to hearing and is necessary for precision diagnostics and ultimately pharmaceutical treatments for hearing loss. Hair-cell survival is not enough to explain the effect of noise on physiological assessments of hearing. These findings highlight the need for more nuanced understanding of the relationship between histopathology and physiology.

SU96. Auditory Neuropathy Associated with Kinase Suppressor of RAS2 Deficiency

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Background: The kinase suppressor of RAS 2 (KSR2) plays a critical role in regulating energy intake, energy expenditure, and substrate utilization. In humans, mutations in the KSR2 locus are associated with severe early-onset obesity and insulin resistance. Studies in Ksr2-knockout (KO) mice demonstrate that Ksr2 expression and function in the hypothalamus are centrally regulated, leading to neuroinflammation, hyperphagia, altered metabolic rates, and consequently obesity and type 2 diabetes (T2D). Among the many mouse genes linked to obesity, Ksr2 deletion produces one of the most profound obese phenotypes, often resulting in early lethality. Obesity, in turn, is inconveniently associated with an elevated risk of sensorineural hearing loss (SNHL), with metabolically unhealthy obesity conferring an even greater risk. A recent meta-analysis confirmed that obesity correlates with a higher prevalence of hearing impairment in adults. In this study, we evaluated auditory function in Ksr2-deficient mice to determine whether obesity driven by Ksr2 deletion contributes to the development of SNHL.

Methods: Ksr2-KO mice on a C57BL/6J-Tyrc-Brd x 129SvEvBrd hybrid background were originally obtained from Lexicon Pharmaceuticals (Woodlands, TX) and transferred to the Veteran's Affairs Loma Linda Healthcare System (VALLHS). The colony was maintained using a heterozygous × heterozygous breeding strategy and housed in a modified Specific Pathogen Free (SPF) facility, without sound conditioning of ambient noise, with ad libitum access to food and water. After weaning, mice were provided a standard diet (Teklad #7001, Envigo) and weighed weekly, while breeders were maintained on a higher-energy diet (Teklad #8626). At approximately 20 weeks of age, hearing function in littermate mice of both genotypes, Ksr2-KO (Ksr2^{-/-}) and wild-type (Ksr2^{+/+}), was assessed using auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE).

Results: As they entered adulthood, Ksr2-KO mice rapidly developed obesity, whereas sex-matched littermates gained weight more gradually and often reached a plateau. By 20 weeks of age, the body weight of Ksr2-KO mice was nearly double that of their littermate controls. DPOAE responses showed deterioration at high-frequency regions with substantial inter-animal variability; however, overall responses were comparable between Ksr2-KO and wild-type mice. Similarly, ABR thresholds did not differ significantly between genotypes. Notably, wave-I ABR amplitudes were markedly reduced in Ksr2-KO mice for both click ($p < 0.0001$) and tonal stimuli ($p < 0.001$, two-way ANOVA).

Conclusions: Although Ksr2-KO mice exhibited a severe obese phenotype, their DPOAE responses and ABR thresholds remained normal, indicating preserved outer hair cell function. In contrast, the significant reduction in wave-I ABR amplitude suggests impaired synaptic transmission between inner hair cells and spiral ganglion neurons, consistent with classic auditory neuropathy. Similar to peripheral neuropathy—which is common in obesity- and T2D-associated neuropathies and typically affects the hands and feet—auditory neuropathy also represents a prevalent form of neuropathy in obesity and T2D populations.

SU97. Sparse Labeling of Spiral Ganglion Neurons Reveals Differences in Spike Initiation Zone Distances by Synaptic Position

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Background: Cochlear hair cells transduce auditory stimuli and send this information to the central nervous system through spiral ganglion neurons (SGNs). The dendrites of type I SGNs are typically unbranched and contact a single inner hair cell. The position of the synapse along the inner hair cell is correlated with synaptic morphology, sound intensity sensitivity, transcriptional profile, and the ion channel properties of post-synaptic type I SGNs. However, the extent to which diversity in SGN output reflects contributions from presynaptic hair cell machinery, intrinsic neuronal properties, or dendritic attenuation remains unclear. To address the latter, we measured the distance between synaptic terminals and spike initiation zones and will present a preliminary model that integrates these measurements with our prior ion channel characterizations as a foundation for a whole-neuron model of Type I SGNs.

Methods: To identify the spike-initiation zone and dendritic length on individual neurons, SGNs were sparsely labeled through two viral transfection strategies. First, an AAV that drives the expression of Cre recombinase was delivered intracerebroventrically in postnatal day 1-3 (P1-3) Ai14 (floxed tdTomato) mice at doses that drive sparse neuronal expression of tdTomato. Second, to selectively label modiolar-contacting SGNs, a Cre-dependent GFP virus was injected into the posterior semicircular canals of P1–P3 NF107Cre mice. Four weeks after injection, cochleae from both ears were collected and immunostained for confocal imaging of fluorescent dendrites and Caspr, a paranodal structural protein marking spike initiation zones.

Results: Across 83 modiolar-contacting and 46 pillar-contacting type I SGNs, we found that modiolar-contacting neurons had significantly shorter dendrite lengths from the peripheral terminal to the Caspr-labeled spike initiation zone. On average, modiolar-contacting dendrites were around 20% shorter than their pillar-contacting counterparts. These findings were repeated

in mice where GFP was expressed only in NF107Cre-positive neurons. 59 out of 68 NF107Cre; GFP positive neurons synapsed on the modiolar side of the inner hair cell. Consistent with these neurons being ‘modiolar’ contacting, these results closely match the proportion of Calretinin-negative NF107Cre; Ai14 somata in the spiral ganglion (223 out of 242).

Conclusions: Whether a neuron successfully fires an action potential depends on many factors, including the expression of ion channels and dendritic morphology. Our goals are to disentangle how anatomical and biophysical factors jointly determine the functional output of SGNs. These results highlight sparse labeling and the NF107Cre line as valuable tools for tracking dendritic lengths and selectively targeting modiolar-contacting type I SGNs. Ongoing work will integrate these data with previous biophysical characterizations of ion channels to assess the relative contributions of dendritic morphology and ion channel properties to the cellular mechanisms underlying SGN subtype diversity.

SU98. Aberrant JAK2 Activation During Development Alters Auditory Thresholds, Neural Gain, and Postsynaptic Architecture

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Background: The JAK-STAT pathway is a central regulator of cytokine and growth factor signaling, with well-established roles in hematopoiesis and inflammation. However, its role in auditory development remains largely unexplored. Aberrant JAK2 signaling is known to drive hematologic disorders and alter immune cell function, raising the possibility that it may also influence cochlear maturation and neural encoding. We investigated how constitutive activation of JAK2 (Jak2CA/WT Pax2Cre) impacts auditory function, outer hair cell (OHC) physiology, spiral ganglion neuron (SGN) subtypes, and synaptic organization.

Methods: Jak2CA/WT Pax2Cre⁺ (EXP) and Jak2CA/WT Pax2Cre⁻ (CTRL) mice were assessed at P28-30. Auditory brainstem responses (ABRs) measured thresholds, P1 latency, intensity, and P1-N1/P3-N3 amplitude intensity functions across 4 - 64 kHz. Distortion product otoacoustic emissions (DPOAEs) were used to evaluate outer hair cell function. Cochlear histology included spiral ganglion neuron (SGN) subtype labeling (Tuj1, Calb1, Calb2, Peripherin), synaptic ribbon - postsynaptic pairing (CtBP2/PSD95), and afferent innervation (neurofilament, myosin 7A).

Results: EXP mice exhibited elevated ABR thresholds across the frequency spectrum, indicating a broad deficit in auditory sensitivity. Latency intensity analysis revealed significant conduction delays at 16 and 32 kHz. Although P1-N1 amplitudes were preserved, higher-order P3-N3 amplitudes were reduced at 5.6-32 kHz, suggesting impaired central auditory gain. DPOAEs demonstrated elevated thresholds and diminished growth functions, particularly at mid-to-high frequencies, consistent with compromised OHC function. SGN analysis showed a significant reduction in Calb2-positive neurons in the apical turn, while Tuj1-, Calb1-, and Peripherin-positive subtypes were preserved, pointing to selective vulnerability of a neuronal population important for temporal encoding. Synaptic ribbon counts and CtBP2/PSD95 pairing were largely intact, but PSD95 puncta appeared globular and disorganized in EXP mice, suggesting altered postsynaptic maturation rather than frank synapse loss. Afferent fiber density and gross

innervation patterns were preserved, supporting the conclusion that functional deficits arise from subtle postsynaptic and neuronal subtype changes rather than widespread neurodegeneration.

Conclusions: Constitutive JAK2 activation disrupts early auditory development at multiple levels. Physiologically, EXP mice show global threshold elevation, conduction delays, impaired OHC function, and reduced higher-order amplitude growth, reflecting deficits in both peripheral sensitivity and central gain. Structurally, these deficits are accompanied by selective SGN subtype loss and altered postsynaptic organization, despite preservation of overall ribbon counts and afferent innervation. These findings identify JAK2 as a novel regulator of cochlear development and neural encoding. Importantly, they suggest that JAK2-targeted therapies, now widely used in hematologic disease, may have unanticipated consequences for auditory function.

SU99. Angiotensin Receptors as Targets for Protection From Noise-Induced Hearing Loss

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Background: The renin–angiotensin–aldosterone system is a ubiquitous endocrine system with significant contributions to inner ear physiology. In opposition to angiotensin II binding to its type 1 receptor (AT1R), which drives inflammation and fibrosis, binding of the type 2 receptor (AT2R) promotes vasodilation, anti-fibrotic, and neuroprotective effects. We hypothesized that AT2R antagonism would impair recovery of auditory function after noise-induced hearing damage, and that conversely, AT1R antagonism using losartan would protect against hearing loss.

Methods: Thirty-two mice (16 male, 16 female) were randomized into four groups of 8. AT1R antagonism was achieved with losartan-infused chow, while AT2R antagonist PD123319 was delivered via continuous subcutaneous infusion via osmotic mini-pump. Controls received standard chow and/or subcutaneous saline. Groups were as follows:

- A: AT1R and AT2R antagonism (losartan + subcutaneous PD123319)
- B: AT1R antagonism (losartan + subcutaneous saline)
- C: AT2R antagonism (control chow + subcutaneous PD123319)
- D: Control chow and subcutaneous saline

Surgical subcutaneous placement of Alzet osmotic mini pumps was performed 1 day prior to noise exposure, and losartan treatment was initiated immediately following noise exposure. Auditory brainstem responses (ABR) were measured at 8, 16, and 32 kHz from 90–10 dB in 5 dB steps at baseline and at 1-, 7-, 14-, and 30-days following noise exposure (8-16kHz octave band noise, 114dB SPL for males, 116dB SPL for females). Experimenters were blinded to group assignment. One-tailed Mann–Whitney U tests were performed to compare elevated post-noise ABR thresholds of select groups.

Results: As compared to baseline levels, noise overexposure resulted in permanently elevated ABR thresholds in all groups, as expected. By day 7, Group C (AT2R inhibitor drug alone) exhibited significantly worsened thresholds at 8 kHz compared with controls in Group D (median 85.0 vs 55.0 dB, $p = 0.0105$), with persistent differences at day 14 (82.5 vs 55.0 dB, $p =$

0.0177) and day 30 (75.0 vs 50.0 dB, $p = 0.01235$). In contrast, AT1R inhibition in Group A (Losartan plus AT2R inhibition) compared to Group C (AT2R inhibition alone) attenuated the noise-induced ABR shift in a protective manner at 8, 16, and 32 kHz across all time points after day 1, except for 8 kHz at day 30 and 32 kHz at day 14. For all comparisons, the magnitude of differences was diminished at higher frequencies.

Conclusions: Type 1 and Type 2 angiotensin receptors confer opposing effects in our mouse model of noise-induced hearing loss. Availability of AT2Rs for activation may provide endogenous protection against worse hearing outcomes during acoustic trauma. In the absence of AT2R signaling, pharmacologic AT1R inhibition with losartan results in significant levels of protection against severe noise toxicity. AT1R inhibition and AT2R activation may represent overlapping strategies for achieving hearing protection against loud noise.

SU100. Hair-Cell Degeneration in Hpsc-Derived Inner-Ear Organoids Carrying TMPRSS3 Variants

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Background: Mutations in TMPRSS3 cause congenital or postlingual hearing loss (DFNB8/10), and defective Tmprss3 proteins are linked to hair-cell loss and altered endocochlear potential. Complex genotype–phenotype correlations in patients with TMPRSS3 variants, and poor cochlear-implant outcomes in some, have impeded our understanding of and treatment plan for TMPRSS3-associated recessive deafness. Since gene therapy has shown promise for treating hearing loss in aged mice with Tmprss3 mutations, we developed a human induced pluripotent stem cell (hiPSC)–derived inner-ear organoid model from TMPRSS3 patients to study the effects of patient-specific mutations on human hair cells.

Methods: hiPSC lines were reprogrammed from blood samples of patients carrying a) a homozygous c.208delC variant and b) compound heterozygous c.1333G > A and c.1273G > A variants; the former had congenital hearing loss, whereas the latter developed postlingual hearing loss. Inner-ear organoids were generated from mutant hiPSC lines and from their isogenic control lines.

Results: Organoid growth and hair-cell development were monitored by immunocytochemistry (ICC) using SOX2 and MYOSIN-7A antibodies. Hair-cell loss was assessed by ICC of cleaved caspase-3 to define the timepoint at which hair-cell degeneration occurs.

Conclusions: We established patient-derived inner ear organoids carrying TMPRSS3 variants for determining the timeline of hair cell degeneration, as well as the role of TMPRSS3 in the human inner ear. This model system will be critical for developing gene therapies for human inner ear disorders.

SU101. Single-Cell RNA Sequencing Reveals Hypoxia-Mediated Transcriptomic Alterations in Vestibular Schwannoma

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Background: Vestibular schwannomas (VS) are benign tumors of the vestibulocochlear nerve, most commonly driven by inherited or somatic mutations in the NF2 gene. Despite their benign histology, VS frequently cause debilitating symptoms, including progressive hearing loss and vestibular dysfunction. Like many solid tumors, VS exhibit regions of intra-tumoral hypoxia. Hypoxia triggers a transcriptional program mediated largely by hypoxia-inducible factor 1 α (HIF-1 α), which has been linked to tumor progression, treatment resistance, and poor outcomes across a variety of cancers. In VS, HIF-1 α expression has been detected, and its inhibition has been shown to suppress tumor growth, suggesting that hypoxia may play a critical role in tumor progression. However, while prior studies have implicated hypoxia in VS progression, the broader impact of hypoxia on the tumor microenvironment and transcriptomic profiles of VS has not been characterized. In this study we aim to investigate how hypoxia influences cellular heterogeneity and gene expression patterns in primary VS cells and identify pathways that may be related to tumor growth and survival.

Methods: Patient-derived VS tumor chunks were enzymatically dissociated and cultivated in proliferative media at room air (21% O₂) and hypoxia (1% O₂). Cells were imaged using light microscopy every week for ~1 month. Cell cultures were then dissociated into single-cell suspensions and subjected to single-cell RNA sequencing (scRNA-seq) using the 10x Genomics Chromium platform. Libraries were prepared using the Chromium Single Cell 3' reagent kit and sequenced on an Illumina platform. Raw data were processed using the Cell Ranger pipeline to generate gene-cell count matrices. Downstream analyses were performed in Seurat, including quality control, normalization, dimensionality reduction, clustering, and differential expression analyses. Immunocytochemistry (ICC) was performed for Schwann cell markers.

Results: Across both conditions, scRNA-seq captured transcriptional profiles from 14,770 high-quality single cells. We identified distinct cellular subpopulations, transcriptional profiles, and enriched pathways between 21% and 1% O₂. The expression of Schwann cell markers will be measured using ICC.

Conclusions: We performed single-cell transcriptomic characterization of primary VS cultivated in room air and in hypoxia. Results will demonstrate how hypoxia shapes cell heterogeneity and transcriptomic profiles in VS. Furthermore, findings in this study may identify candidate pathways for future therapeutic treatments for VS.

SU102. Auditory Temporal Responses of Mutant Mice and Diagnosis of Hearing Loss Pathology

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Background: As the search for treatments for hearing impairment gathers pace, it is becoming increasingly important to develop reliable tools to diagnose the underlying pathology so that the appropriate treatment method may be delivered. We use mutant mice to aid the development of non-invasive physiological tests that may be transferrable into a clinical setting. These mice carry different single gene mutations and have known sites of lesion covering the three major

categories of sensorineural hearing impairment described by Schuknecht and Gacek (1993); Sensory (hair cell dysfunction), Metabolic (stria vascularis dysfunction), and Neural (auditory neuron defects).

Methods: Amplitude Modulation Following Responses (AMFR) were evoked by stimuli comprised of 12kHz (or 24 kHz) carrier tones presented at 80dB SPL (100ms duration, 5ms rise/fall time), which were 100% sinusoidally amplitude-modulated at rates (modulation frequency, fm) from 100-1750Hz, and by presentation of 1000 Hz fm across stimulus levels from 0 – 95 dB SPL. We calculated AMFR magnitude, phase, group delay and synchrony coefficient as a function of fm or stimulus level (eg Shaheen et al. 2015). Auditory Brainstem Responses (ABRs) and thresholds were recorded in the same mice.

Results: AMFR amplitude vs fm transfer functions recorded at 80dB SPL were typically multi-peaked in normal hearing littermate control mice, having peaks with a range close to modulation rates of 300 Hz, 600 Hz and 1000 Hz. *Klhl18* < lowf > (Inner Hair Cell dysfunction), *Mir96* < tm3.1Wtsi > (Inner and Outer Hair Cell dysfunction), *Slc26a5* < tm1-Cre > (Prestin, OHC dysfunction), and *Wbp2* < tm2a > (dysfunctional innervation) mutant mice all showed pronounced reductions in AMFR amplitudes and synchrony at these three fm ranges. *Sgms1* < tm1b > and *Slpr2* < stdf > mutant mice (both with a stria vascularis dysfunction resulting in a reduced endocochlear potential) showed reduced AMFR amplitudes and synchrony across a more limited span of fm in the 300 Hz and 600 Hz ranges, but in contrast to the other mutants examined, these AMFRs were almost indistinguishable from littermate controls at the higher modulation rates tested. AMFR amplitudes recorded at the lower and mid-range modulation rates across all mutant mice were correlated with the auditory brainstem response thresholds of these mice. However, at the higher modulation rates, amplitudes recorded from strial mutant mice were more comparable to controls. Typically, across all pathologies, mice with higher ABR threshold generated lower amplitude AMFR, and those with lower ABR thresholds generated higher amplitude AMFRs.

Conclusions: Reduced AMFR amplitudes in hearing-impaired mice may be a general feature across “neural” and “sensory” pathologies, in contrast to the normal AMFR amplitudes seen at higher modulation rates in “metabolic” impairment. These observations may help in the development of a non-invasive physiological diagnostic test battery to differentiate different sites of pathology underlying inner ear sensorineural hearing loss and help to inform better treatment strategies for individuals.

SU103. A Simplified Human Full-Head Model for Investigating Electric-Acoustic Interaction With Intra- and Extra-Cochlear Electric and Acoustic Stimulation

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Background: Computational models of auditory nerve activity constitute indispensable tools for elucidating the neural mechanisms of sound perception. They enable systematic investigation of

the complex interactions between electric and acoustic stimulation (EAS) within the auditory system. A computational model has been substantially extended to represent more accurately the anatomical and electrical properties of the full human head. The extended model incorporates detailed 3D representations of the outer, middle, and inner ear, as well as the brain, forming a population-based full-head model. The model can be used to simulate intra- and extra-cochlear electric stimulation of the auditory system as well as the interactions between electric and acoustic stimulation. The goal of the computation model is to optimize the amount of current flow inside the cochlea by varying the location of the stimulating and reference electrode and assess interactions between electric and acoustic stimulation.

Methods: Finite element method (FEM) simulations were conducted under monopolar stimulation modes for both intra- and extra-cochlear electrodes. A novel computational framework was implemented to simulate both acoustically evoked compound action potentials (aCAPs), electrically evoked compound action potentials (eCAPs) and Electrocochleography (ECochG) in humans, with a specific focus on interactions arising from combined electric-acoustic stimulation (EAS) at the level of the auditory nerve. Key variables simulated include: transimpedance matrices (TIMs) of intracochlear electrodes, intra- and extra-cochlear voltages and currents, auditory nerve activity, and the resulting aCAPs, eCAPs and ECochG.

Results: The model reliably reproduced transimpedance matrices (TIMs) and response trends consistent with those reported in cochlear implant users with the same electrode array. Novel intra- and extra-cochlear current pathways through the scala tympani were identified, revealing distinct voltage and current density distributions between the two stimulation modes. The framework is capable of simulating electrocochleography (ECochG) responses resembling human recordings and providing a complementary means to assess electric-acoustic interactions. Preliminary simulations confirmed measurable interaction effects at hair cell and auditory nerve level. Simulated compound action potential (CAP) waveforms from intracochlear electrodes closely matched those observed in human recordings. A marked reduction in eCAP responses was observed under both intra- and extra-cochlear stimulation when an acoustic masker was applied. Mechanisms underlying EAS interactions in CAP responses were systematically investigated, with spike interactions in the apical cochlea and ectopic stimulation of the auditory nerve trunk emerging as the most plausible explanations.

Conclusions: This simplified full-head human model represents a promising tool for investigating electric-acoustic interactions and optimizing electric stimulation for both intra- and extra-cochlear electric stimulation in cochlear implant users. These results are relevant to create a diagnostic to diagnose hearing loss and a prosthetic device that augments acoustic hearing with extra-cochlear electric stimulation.

SU104. ADARB1 as a Nuclear Marker of Spiral Ganglion Neurons Across Species and Age

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Background: Spiral ganglion neurons (SGNs) are the primary neurons responsible for transmitting auditory signals from the inner ear to the brain. Degeneration of SGNs occurs in various conditions including auditory neuropathies, traumatic brain injury, and aging, thus

contributing to acquired hearing loss. Precise characterization and quantification of SGNs are critical for advancing the understanding of auditory pathology and evaluating potential therapeutic interventions. Here we show that immunolabeling for ADARB1, an RNA editing enzyme enriched in the nuclei of glutamate receptor-expressing neurons, is a reliable method for SGN identification and quantification in young adult mice. We compared manual SGN counts obtained via neurofilament heavy chain (NFH) immunolabeling to automated counts generated with ADARB1 immunofluorescence. We further characterized ADARB1 immunoreactivity in aged mice (24 months), as well as in human and macaque inner ear tissues. Co-labeling sections for ADARB1 and HuD, NFH, GATA3, or SOX2 was undertaken to confirm neuronal identity.

Methods: Mid-modiolar cochlear sections were obtained from 3-week-old CD1 mice, 24-month-old C57Bl/6 mice, and archived (> 5 years) temporal bone sections from macaques (4 years-old) and human cadavers of varying ages (all > 40 years of age). The human, macaque, and 24-month-old mouse samples were immunolabeled with antibodies against ADARB1 and neurofilament heavy chain (NFH), while the 3-week-old mouse samples were immunolabeled with antibodies against ADARB1, NFH or HuD, and either SOX2 or GATA3. Micrograph images were acquired using laser scanning confocal microscopy with a 40X objective. Image processing, including manual thresholding and noise reduction, was performed using ImageJ (Fiji).

Results: Within Rosenthal's canal, ADARB1 immunolabeling yielded specific and robust staining of spiral ganglion neurons (SGNs), allowing for reliable automated quantification using the analyze particles function in ImageJ. Comparison across over 30 sets of images yielded a Lin's concordance correlation coefficient > 0.95, indicating strong agreement between manual and automated counts. Strong ADARB1 expression was observed in surviving SGNs in 24-month-old mouse samples demonstrating utility in a context where hair cell and SGN loss were evident. ADARB1 labeling of SGN nuclei was also prominent in macaque and human sections, though some variability was noted in the cadaveric samples. Colocalization analyses demonstrated that ADARB1 labels both type I and type II SGNs, as evidenced by overlap with HuD, NFH, and GATA3-labeling. Conversely, a lack of colocalization between ADARB1 and SOX2 indicated minimal ADARB1 expression in glial cells.

Conclusions: These studies demonstrate the specificity and robust expression of ADARB1 in spiral ganglion neurons (SGNs) and validate the effectiveness of ADARB1 labeling for automated quantification of SGNs, indiscriminate of type, and across diverse mammalian species.

SU105. Reimbursement for Laryngology Procedures Performed in Facility-Based Versus Non-Facility-Based Settings: Trends and Implications for Practice

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Background: The Centers for Medicare and Medicaid Services (CMS) create a method of payment for enrolled healthcare providers called the Medicare Physician Fee Schedule (MPFS). Procedures have separate reimbursement rates for physician services provided in facility and non-facility-based settings. When procedures are performed in facility-based settings, CMS

reimburses a technical fee and allows devices to be billed for separately from the procedure (e.g., laser fibers and balloons.) Conversely, devices used in non-facility-based settings cannot be billed separately from the procedure CPT code; thus, when assessing profitability, the cost of these is subtracted from the total reimbursement. Hence, device cost can be prohibitive in performing these procedures in the non-facility-based setting, forcing procedures to be done in higher-cost locations such as the operating room (OR) or surgery center. There are limited studies examining reimbursement patterns in these two different practice settings. Thus, this study compares the reimbursement rates of commonly performed office-based Laryngology procedures, considering the cost of devices, fillers, and pharmaceuticals, for facility-based versus non-facility-based settings.

Methods: The MPFS Look-Up Tool was used to determine the reimbursement for 11 common office-based Laryngology clinic procedures for facility-based and non-facility-based locations. The technical fee for the facility-based setting was provided by a CMS billing consultant. The cost of pharmaceuticals, devices, or fillers was subtracted from the reimbursement for each procedure to determine the profit margin per procedure, which was calculated for all procedures.

Results: Procedures requiring balloon devices, injection augmentation materials, and laser fibers in the non-facility setting reimbursed significantly lower (76.5%, 62.6 – 95.9%, and 99.7% lower, respectively) compared to facility settings that receive both technical and professional fee reimbursements ($p < 0.05$). When accounting for procedural materials (e.g., injectable filler), profit margins were considerably lower in the non-facility setting (40.5%, 4.8 – 60.6%, and 2.3% relative profit margins, respectively.) All other procedures were profitable in both settings but more so in the facility setting (52.5 – 87.3% reductions in reimbursement in non-facility versus facility settings.)

Conclusions: Overall healthcare utilization cost of Laryngology procedures in a clinic setting is favorable to an OR setting, but clinicians who practice in non-facility-based clinics are at risk for a monetary loss due to lower reimbursements and expensive devices. Performing office-based procedures is an important component in providing equitable and timely treatment. If reimbursement rates continue to bias in favor of facility-based settings, there is a risk of non-facility-based clinics no longer performing procedures or of increasing overall healthcare costs by shifting them to the OR setting.

SU106. Effect of IL-8 Inhibition on Cancer Cell Proliferation and Migration in Oral Cavity Squamous Cell Carcinoma

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Background: Oral cavity squamous cell carcinoma (OCSCC) is the most common SCC of the head and neck and the 6th most common cancer worldwide. A significant poor prognostic factor in OCSCC is perineural invasion (PNI), which occurs in an estimated 24-31% of cases and can lead to taste alterations. PNI is the spread of cancer into or around nearby nerves, and when present, is associated with higher locoregional recurrence and worse disease-free and overall survival. The mechanisms underlying PNI is still unresolved, however recent evidence suggest

that both Schwann cells and cancer secrete IL-8, which may have a role in promoting OCSCC cell migration and proliferation. We hypothesize inhibiting IL-8 signaling in OCSCC cells will reduce cancer cell proliferation and migration.

Methods: Human OCSCC (SCC9, UCSFOT1109) and Schwann cell lines (HS11, FHSC) were cultured in maintenance media. Cell-conditioned supernatants were collected at 48 hours, and antibody arrays were utilized to profile the secretion of 80 cytokines. A top secreting cytokine was determined to be interleukin-8 (IL-8). Subsequently, OCSCC cells were cultured in serum-reduced conditions and exposed to 0 or 1000 ng/ml anti-IL-8 neutralizing antibody for 12, 24, and 48 hours. Transwell and scratch assays were performed to measure the effect of anti-IL-8 on chemotaxis and cell migration. Viability assays were utilized to determine the impact of anti-IL-8 on cell proliferation.

Results: IL-8 was highly secreted by SCC9, UCSFOT1109, HS11, and FHSC. Anti-IL-8 antibody significantly reduced gap closure on scratch assay in both OCSCC cell lines at 4 hours; however, the effect did not persist at longer time points. Additionally, on transwell assay, anti-IL-8 antibodies also significantly reduced the number of migrated cells at all time points in SCC9 and early point of 12 hours in UCSFOT1109. Anti-IL-8 antibody did not significantly affect the proliferation of either OCSCC cell lines at any time point.

Conclusions: We found that OCSCC and Schwann cells secrete high levels of IL-8 into the tumor-nerve microenvironment. Inhibiting IL-8 signaling in vitro using anti-IL-8 neutralizing antibodies reduced migration, but did not affect proliferation, of OCSCC. Further investigation into the role of IL-8 in the tumor-nerve microenvironment may provide additional insights into cancer spread and perineural invasion and may reveal novel therapeutic targets for treatment of OCSCC.

SU107. Hypoxia-Induced BNIP3 and HIF-1 α Reveal Metabolic Vulnerabilities in Vestibular Schwannoma

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Background: Under hypoxic conditions, cancer cells can undergo metabolic reprogramming through the induction of hypoxia-inducible factors (HIF-1 α) and Bcl-2/adenovirus E1B 19 kDa interacting protein 3 (BNIP3), enabling them to generate energy and biosynthetic precursors needed for survival. Vestibular schwannomas (VS), benign tumors of the vestibulocochlear nerve, are known to express HIF-1 α , which promotes angiogenesis and tumor growth. However, the metabolic response of VS to hypoxia, and how it compares to wild-type Schwann cells (WT-SCs) and merlin-deficient Schwann cells (MD-SCs), remains poorly understood. This study aims to define how oxygen tension influences metabolism and hypoxia-related signaling across these cell types.

Methods: Primary VS cells were derived from patient tumors, while MD-SCs were generated by introducing NF2 mutations into WT-SCs derived from fetal tissue and human induced

pluripotent stem cells. VS, WT-SCs, and MD-SCs were cultured for 72 hours under normoxia (21% O₂), physioxia (5% O₂), or hypoxia (1% O₂). Metabolic activity was assessed using CellTiter-Glo assays, and immunocytochemistry (ICC) was performed for BNIP3 and HIF-1 α . Data were analyzed with descriptive statistics and one-way ANOVA.

Results: VS cells demonstrated the lowest ATP production at 1% O₂, consistent with reduced oxidative phosphorylation under hypoxia. A majority of VS cultures exhibited robust upregulation of HIF-1 α and BNIP3 at 1% O₂, with concordant expression patterns within samples. In contrast, WT-SCs and MD-SCs did not show significant hypoxia-induced upregulation of these markers.

Conclusions: Most primary VS cells upregulate BNIP3 and HIF-1 α under hypoxia, demonstrating their ability to undergo metabolic reprogramming and adapt to oxygen-limited conditions. In contrast, WT-SCs and MD-SCs did not exhibit this response, suggesting that NF2 loss alone is insufficient to drive these changes. These results highlight hypoxia-driven adaptations as a defining feature of VS biology and point to metabolic pathways as promising therapeutic targets for VS.

SU108. Global Mapping of Clinical Trials in Hearing Disorders (2019–2024): A Systematic Mapping Review

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Background: Hearing disorders pose a significant public health burden, yet no comprehensive mapping of clinical research exists. This project systematically catalogues and analyzes clinical trials over the past five years to identify evidence gaps and redundancies in prevention and treatment strategies in the field.

Methods: We conducted a systematic search of four international trial registries, the US ClinicalTrials.gov registry, the Australian New Zealand Clinical Trials Registry (ANZCTR), the ISRCTN registry in the United Kingdom and the European EU clinical Trials Register and Clinical Trials information system (EUCTR/CTIS), identifying interventional studies first posted between January 1, 2019, to August 14, 2024. Eligible studies evaluated interventions for hearing disorders across all ages and severities. Data were extracted using a standardized PICO (Population, Intervention, Comparison, Outcome) framework, covering study design, populations, interventions, outcomes, sample size, funding, and geographical distribution. Additional study characteristics, including randomization, trial phase, recruitment status, and number of arms, were recorded, along with the results posting and associated publications of completed studies. Two epidemiologists independently screened records (30% duplicate screening for quality assurance). A hierarchical classification system was developed for interventions (pharmacological, devices, gene therapy, etc.) and hearing conditions by laterality,

onset, and severity following WHO criteria and the International Bureau for Audiophonology BIAP Recommendation 02/1).

Results: Of 1,679 identified records, 547 trials met inclusion criteria (67% excluded, primarily for non-interventional designs or off-topic focus). Most were randomized (58.9%), assessed therapeutic interventions (66%), and involved adults (81.6%). Common conditions included sensorineural hearing loss (19.9%), tinnitus (19.2%), and presbycusis (4.3%). Device-based interventions were the most prevalent (37.1%), followed by non-pharmacological therapies (17.1%) and pharmacological treatment (10.3%).

Data on hearing loss characteristics were variably reported. Regarding the type of hearing loss, 9.1% were unilateral, 19.2% bilateral, 11.6% included both types, and 1.6% had normal hearing; 58.5% were not reported.

Only 13.3% of completed trials (n=210) had results posted in registries; 31 were linked to PubMed publications. Geographically, the U.S. (34.7%), France (6.8%), and Australia (6.8%) led research output, with 85.7% of studies being nationally focused. Descriptive statistics and an interactive evidence map were generated.

Conclusions: This first systematic map of hearing disorders' trials reveals disparities in research focus, with underrepresentation of pediatric populations and low publication rates despite trial completion. The interactive dashboard highlights regional and thematic gaps. Such mapping is essential for identifying areas of research that have already been thoroughly explored, thereby limiting duplication, as well as highlighting persistent gaps in the scientific literature. This open-access platform will serve as a data-driven decision-making tool, facilitating the identification of research priorities and optimizing the impact of scientific work in the field of hearing health.

SU109. Incidence and Risk Factors of Postoperative Sensorineural Hearing Loss After Cholesteatoma Removal

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Background: While cholesteatoma and chronic ear disease are primarily associated with conductive hearing loss, all otologic surgical intervention carries a risk of sensorineural hearing loss (SNHL). This study aims to evaluate the incidence of SNHL following cholesteatoma surgery and identify potential risk factors contributing to its development.

Methods: A retrospective chart review was conducted of 345 patients who underwent surgery for cholesteatoma removal between January 2021 and June 2024. Data included patient demographics, prior ossicular reconstruction, prior surgery, extent of surgery, disease characteristics, and audiometric data. Multivariable logistic regression was utilized to assess associations between potential risk factors and the development of SNHL. SNHL was defined as a ≥ 20 dB drop in bone conduction threshold at any frequency from 250-4000Hz on postoperative audiograms ranging from 2-24 months.

Results: 345 patients [mean age 51.2 ± 17.3 years; 187 (54.2%) males] were analyzed. 301 had preoperative mixed or conductive hearing loss, mean air-bone gap (ABG) was 26.1 dB, and 182

(61.7%) demonstrated postoperative improvement in the ABG (mean 21.3 dB). 59 patients (17.1%) developed postoperative SNHL, with 56 patients (16.2%) demonstrating SNHL within the first year. 3 patients (0.9%) presented with SNHL at 12-24 months. Prior ossicular prostheses (OR 3.74 [1.08, 13.2]; $p=0.037$) was associated with increased risk of postoperative SNHL, whereas new prosthesis placement (OR 0.19 [0.055, 0.545]; $p=0.0041$) and a canal wall down approach (OR 0.268 [0.110, 0.614]; $p=0.0026$) was associated with a decreased likelihood when controlling for race and surgeon. Revision surgery, age, sex, and race did not reach significance. The 17.1% incidence of SNHL, primarily in the first year, constitutes a clinically meaningful risk profile that should inform prognostic counseling and follow-up planning.

Conclusions: These findings provide contemporary incidence and risk profiling relevant to otologic surgical planning, prognosis, and patient expectation setting. SNHL is a non-negligible complication after cholesteatoma surgery, with patients' risk profiles influenced by a prior and current prosthesis placement as well as the extent of surgical intervention. Key takeaways include: (a) postoperative SNHL > 20 dB at any frequency after cholesteatoma surgery is not rare (~1 in 6); (b) most cases appear within 12 months; (c) prior ossicular prosthesis is a salient risk marker; and (d) in this cohort, canal wall down approach and new prosthesis placement correlate with lower odds of developing postoperative SNHL. While canal wall down approach generally entails a more extensive surgery, this association may reflect technique-mediated protection (e.g., less ossicular manipulation) or be explained by greater rates of preoperative SNHL (i.e., confounding by indication). Future research with larger patient cohorts may help optimize risk stratification, identify causal pathways, and develop hearing preservation protocols to enhance patient counseling and surgical management.

SU110. The Role of Concomitant Medications in Improving SPI-1005 Study Outcomes in Meniere's Disease Patients in a Phase 3 Randomized Placebo-Controlled Trial (STOPMD-3)

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Background: Meniere's disease (MD) involves episodic vertigo or dizziness, fluctuating low frequency hearing loss, and intermittent or constant tinnitus. No FDA-approved drug therapies exist for MD or its auditory or vestibular symptoms. Medical management with diuretics, betahistidine, and corticosteroids have demonstrated low evidence of efficacy. SPI-1005, a novel anti-inflammatory drug, has shown safety and efficacy in three MD studies: Phase 1b (N=39), Phase 2b (N=126), Phase 3 (N=221) randomized clinical trials (RCTs) after 21 or 28-days of oral treatment. In the Phase 3 RCT, SPI-1005 treatment showed significant improvements in two pre-specified primary efficacy endpoints involving low-frequency hearing and speech discrimination over placebo. Here, we present post-hoc analyses of the Phase 3 RCT involving common concomitant medications (CM) used to manage MD.

Methods: Adults (18-75 years) were enrolled at 11 US sites (NCT04677972) and were randomized to either SPI-1005 400 mg or placebo BID, for 28 days. Participants were allowed to continue existing CM, excluding corticosteroids. Pure-tone audiometry (PTA), words-in-noise (WIN), tinnitus-functional-index (TFI) including VAS-loudness (TL), vertigo-symptoms-scale (VSS), dizziness-handicap-inventory (DHI), and VAS-aural fullness (AFS) were assessed at

baseline, 28, 56, and 84-days. Compliant participants entered OLE and received SPI-1005 400 mg BID, for up to 12-months, and were reassessed every 3-months. Improvements in low-frequency PTA (LFPTA) and WIN scores in either ear from baseline were applied: ≥ 10 dB at 250, 500, or 1000 Hz; and ≥ 4 -words in WIN at 84-days. CMs taken by $> 15\%$ of participants were identified and stratified.

Results: 191 Per-Protocol subjects (mean age 55.5; 103 males/88 females) were analyzed. CM use was 94 (49.2%) for hydrochlorothiazide, 74 (38.7%) for triamterene, 44 (23.0%) for hydrochlorothiazide/triamterene, 38 (19.9%) for diazepam, 35 (18.3%) for betahistine, and 34 (17.8%) for meclizine.

LFPTA improvements for SPI-1005 vs placebo with hydrochlorothiazide (27/48, 56.3%, vs 15/46, 32.6%, $p=0.0243$); triamterene (22/36, 61.1%, vs 12/38, 31.6%, $p=0.0190$); hydrochlorothiazide/triamterene (12/17, 70.6%, vs 8/27, 29.6%, $p=0.0128$); diazepam (12/16, 75.0%, vs 9/22, 40.9%, $p=0.0516$); betahistine (9/18, 50.0%, vs 7/17, 41.2%, $p=0.7380$); meclizine (11/18, 61.1%, vs 5/16, 31.3%, $p=0.1005$).

WIN improvements for SPI-1005 vs placebo with hydrochlorothiazide (21/48, 43.75%, vs 15/46, 32.6%, $p=0.2950$); triamterene (16/36, 44.4%, vs 13/38, 34.2%, $p=0.4757$); hydrochlorothiazide/triamterene (9/17, 52.9%, vs 10/27, 37.0%, $p=0.3588$); diazepam (4/16, 25.0%, vs 7/22, 31.8%, $p=0.7292$); betahistine (8/18, 44.4%, vs 3/17, 17.6%, $p=0.1464$); meclizine (9/18, 50.0%, vs 4/16, 25.0%, $p=0.1717$).

During OLE, subjects treated with SPI-1005 and CM continued to show LFPTA (27-65%) and WIN (18-50%) improvements at 3- to 12-months. No significant differences were found between SPI-1005+CM vs SPI-1005 alone.

Conclusions: Adjunctive therapy with SPI-1005 on to the existing medical management for MD involving diuretics (hydrochlorothiazide, triamterene, or hydrochlorothiazide/triamterene) demonstrated significant improvements in low-frequency hearing but not speech discrimination over placebo. Non-significant improvements were observed for diazepam, betahistine, and meclizine.

SU111. Systemic Ebselen Treatment Prevents Amikacin Induced Ototoxicity and Nephrotoxicity in Mice

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Background: Aminoglycosides (AGs), such as tobramycin and amikacin, commonly used to treat recurrent pulmonary infections, are ototoxic and nephrotoxic. Our previous mouse studies showed that 14-days of amikacin (500 mg/kg/day) induced temporary threshold shifts (1-11 dB), tinnitus (24%), and hyperacusis (82%) which were attenuated by ebselen (20 mg/kg/day). The present study determined whether 28-days of amikacin increases the severity/duration of ototoxicity and if ebselen prevents amikacin-induced ototoxicity and nephrotoxicity.

Methods: Adult CBA/CaJ mice (N=32) 3-7 months old were divided into three groups: 1) Control (N=10); 2) Amikacin (N=10); and 3) Amikacin+Ebselen (N=12). Groups 2 and 3 received vehicle or ebselen (20 mg/kg/day x 28-days i.p.) followed by amikacin (500 mg/kg/day

x 28-days s.c.) 30-minutes later, respectively. Auditory brain-stem response (ABR) and acoustic startle response (ASR) were performed at baseline and at 4-, 8-, 12-, and 16- weeks from the start of treatment. ABR was also performed at 35-weeks. Clinically relevant change (CRC) using ABR threshold shift criteria (hearing loss), gap-induced-prepulse-inhibition of the acoustic startle changes (tinnitus), and startle magnitude shifts (hyperacusis) were analyzed. Cochlear histopathology was performed using Myosin-7a immunolabeling to identify outer hair cell (OHC) loss. Serum samples were collected at baseline and 4-weeks for blood urea nitrogen (BUN) and creatinine (Cr) levels (nephrotoxicity).

Results: Control mice showed permanent threshold shifts (PTS) of 1–12 dB at multiple frequencies and time-points. CRC rates were 25%, 20%, 15%, 35%, and 65% at 4-, 8-, 12-, 16-, and 35-weeks, respectively. Tinnitus incidence was 0%, 0%, 0%, and 10% at 4-, 8-, 12-, and 16-weeks, respectively with no observed hyperacusis.

28-day amikacin resulted in PTS of 14–25 dB at multiple low frequencies and time-points. CRC rates were 75%, 60%, 45%, 50%, and 94%, respectively. Tinnitus incidence was 22%, 22%, 33%, and 0%, respectively with no hyperacusis.

28-days ebselen significantly reduced amikacin-induced PTS to 4–20 dB at multiple low frequencies and time-points: 4-weeks ($p=0.0029$) and 8-weeks ($p=0.0032$). CRC rates were also reduced to 29% ($p=0.0056$), 8% ($p=0.0003$), 33%, 46%, and 79%, respectively. Tinnitus incidence was 13%, 25%, 0%, and 13%, respectively with no hypoacusis.

Ebselen prevented amikacin-induced changes in Cr levels at 4-weeks, -0.042 vs $+0.030$ mg/dL ($p=0.0216$). BUN levels did not significantly change from baseline levels in either group.

Conclusions: 28-days of amikacin increased the severity and duration of ototoxicity that was significantly reduced by ebselen. Amikacin-induced nephrotoxicity (increased Cr) was significantly reduced by ebselen. Interestingly, no hyperacusis was observed in any group and may be a consequence of greater PTS. These findings are consistent with recent clinical data from a Phase2-RCT involving adults receiving 14-days of IV-tobramycin. These results extend our earlier findings involving 14-days of amikacin using a dose schedule that mimics the clinical treatment of multi-drug resistant TB and NTM.

SU112. Furosemide Attenuates Noise-Induced Hearing Loss and Potentiates Preventive Effect of N-Acetylcysteine

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Background: Noise-induced hearing loss (NIHL) is a major cause of sensory hair cell damage, primarily driven by dysregulation of reactive oxygen species (ROS) homeostasis. Antioxidants such as N-acetylcysteine (NAC) have demonstrated protective effects, underscoring the role of ROS in NIHL. However, no FDA-approved pharmaceutical treatment currently exists, partly due to the complexity of cochlear damage mechanisms and the restrictive nature of the blood-labyrinth barrier (BLB), which limits drug delivery to the inner ear. This study investigated the protective mechanisms of furosemide (FRS) against NIHL and its potential to enhance the efficacy of NAC.

Methods: FVB/NJ mice were pretreated with a single dose of FRS (200 mg/kg) prior to noise exposure. Auditory brainstem response (ABR) thresholds and endocochlear potential (EP) were measured to assess auditory function. The permeability of the stria vascularis (SV) to small molecules and protein-bound tracers was evaluated. The combined effect of FRS and NAC on outer hair cell (OHC) survival and hearing prevention was also examined.

Results: FRS pretreatment temporarily reduced EP without elevating ABR thresholds and significantly attenuated NIHL. The most effective protection was observed when FRS was administered immediately before noise exposure. FRS selectively increased SV permeability to small molecules, facilitating drug delivery without affecting larger protein-bound tracers. Combined FRS and NAC treatment enhanced antioxidant effects, resulting in additive protection against OHC loss and NIHL, with near-complete preservation of OHCs.

Conclusions: Furosemide effectively attenuates NIHL by transiently modulating cochlear physiology and enhancing drug permeability across the BLB. Its combination with NAC offers a promising strategy for preventing NIHL, highlighting a potential therapeutic approach for clinical application.

SU113. Insulin-Like Growth Factor 1 Protects Cochlear Outer Hair Cells Against Cisplatin by Reducing Oxidative Stress and Inhibiting Apoptosis

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Background: Cisplatin is a widely used and highly effective anti-neoplastic agent included in numerous oncology treatment protocols for both pediatric and adult patients. However, one of its most serious adverse effects is ototoxicity, which currently lacks any clinically approved preventive or therapeutic interventions. Histological studies have shown that cochlear lesions following cisplatin administration are particularly prominent in outer hair cells (OHCs) located in the organ of Corti, contributing significantly to irreversible sensorineural hearing loss and decreased quality of life. Our previous studies demonstrated that insulin-like growth factor 1 (IGF1) has a protective role in the cochlea, promoting the survival of auditory hair cells (HCs) after diverse forms of cochlear injury, including noise trauma, ischemia, surgical insult, and exposure to aminoglycoside antibiotics. These findings prompted us to explore whether IGF1 can protect cochlear sensory HCs against cisplatin-induced toxicity. In this study, we utilized a cochlear explant culture model derived from postnatal day 2 mice to evaluate the otoprotective efficacy of IGF1 against cisplatin exposure.

Methods: To determine the protective effects of IGF1 against cisplatin, the explants grown in the medium containing 10 μ M cisplatin were treated for 36 h with 1 or 5 μ g/ml recombinant human IGF1. To assess whether the protective effects were induced by IGF1 binding to its receptor (IGF1R), the receptor-competitive antagonist or the IGF1R tyrosine kinase inhibitor was used. Apoptosis in the cochlear epithelium was determined by treating the explants with 10 μ M cisplatin with or without 5 μ g/ml IGF1 and immunostaining with anti-cleaved caspase 3 antibodies. The effects on the cell cycle were examined by adding 3 μ g/ml 5-bromo-2'-deoxyuridine (BrdU) to the explants treated with 10 μ M cisplatin with or without 5 μ g/ml IGF1

for 36 h. To determine the antioxidant capacity of IGF1, the glutathione to glutathione disulfide (GSH/GSSG) ratios of the cochlear supernatant were measured using the GSH/GSSG-Glo assay.

Results: Administration of IGF1 to the explants grown in the medium containing cisplatin markedly protected outer HCs from cisplatin-induced damage. Pharmacological inhibition of IGF1R using an IGF1R antagonist or inhibitor markedly attenuated the protective activity of IGF1, indicating that IGF1R is specifically required for IGF1 effects in HCs against cisplatin. As a protective mechanism against cisplatin, we found that the administration of IGF1 reduces cisplatin-induced oxidative stress. IGF1 effects on the maintenance of HC numbers are achieved by inhibiting the apoptosis of HCs, not by inducing the proliferation of HCs or supporting cells (SCs).

Conclusions: These data suggest that IGF1 may serve as a promising and clinically applicable therapeutic strategy for preventing cisplatin-induced ototoxic damage in patients undergoing chemotherapy.

SU114. Predicting the Ototoxic Risk of Fda-Approved Drugs Through Machine Learning and in Vivo Approaches

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Background: The ototoxic potential of drugs is rarely evaluated during pre-clinical development or clinical trials, resulting in hearing loss as an underrecognized adverse effect of many medications in use. Ototoxicity likely remains underreported for numerous FDA-approved drugs due to a lack of predictive tools or mandatory regulation. Our goal is to predict the ototoxic potential of FDA-approved drugs using a machine learning (ML) model and to validate the predictions experimentally in the zebrafish lateral line.

Methods: The ML model was trained on a curated dataset of 283 compounds with established ototoxic or non-ototoxic classifications derived from published literature. This model was based on an F1-optimal threshold, high-recall screening, and well-calibrated probabilities, with flexible risk-tolerance tuning for new compound libraries. We applied the model to a DrugBank database of 2423 FDA-approved drugs, then validated a subset of model predictions in larval Brn3c:mGFP transgenic fish, which express hair cell-specific GFP. The larvae were exposed to various concentrations of predicted ototoxins and compared to vehicle-treated controls, followed by hair cell quantification using fluorescence microscopy.

Results: Our model predicted that among the FDA-approved drugs, 692 compounds have ototoxic potential based on their physicochemical and pharmacological properties. Of the top 60 compounds, 18 were known ototoxins such as aminoglycosides and loop diuretics. From the predicted list, we selected 30 drugs with a model-assigned probability of ototoxicity between 67–100% for experimental validation. Known ototoxins were excluded to focus on compounds previously not associated with auditory toxicity. To date, we have experimentally tested eight compounds and confirmed that two drugs, Dantrolene and Nitrofurantoin, were ototoxic. A third, Fruquintinib, showed hair cell loss only at the highest dose, suggesting general cytotoxicity. Among the 32 evaluated drugs, the model correctly predicted 20 as ototoxic (18 known and 2 experimentally validated), corresponding to 62% alignment with expected outcomes.

Conclusions: Preliminary findings demonstrate that ML-based predictions for unrecognized ototoxic compounds need improvement, as 2 out of 8 tested drugs were confirmed as ototoxins in zebrafish. Further experiments will explore whether the confirmed ototoxic drugs from the predicted list enter via mechanotransduction (MET) channels, affect ribbon synapses, and ultimately impact hair cell function. Our future work will focus on refining the model to achieve > 90% prediction confidence and developing it into an accessible, robust screening tool for drug-induced ototoxicity.

SU115. Prevention of Hearing Loss in the Cochlea of Type 2 Diabetes Model Mice

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Background: The number of patients with diabetes mellitus is increasing worldwide due to changes in lifestyle and social environment. Diabetic patients are more likely to develop hearing loss (diabetes-related hearing loss), which often occurs from a relatively young age and significantly reduces quality of life (QOL). To clarify the mechanisms and effective preventive strategies of diabetic hearing loss, we investigated auditory function, cochlear histopathology, and preventive interventions using a type 2 diabetes mouse model.

Methods: TSOD mice, a type 2 diabetes model, and control mice were used. Auditory function was evaluated periodically by auditory brainstem response (ABR). After evaluation, cochleae were harvested from the temporal bone, and vascular structures were examined by vascular staining along with assessment of hair cells and spiral ganglion cells. Preventive interventions included caloric restriction, administration of traditional Japanese herbal medicines (Bofutsusho-san and Dai-saiko-to), metformin, and SGLT2 inhibitors.

Results: TSOD mice exhibited hyperglycemia, obesity, and earlier onset of hearing loss compared with controls. In the cochlea, reduced capillary area was observed in the stria vascularis. Preventive interventions showed that caloric restriction and herbal medicine administration were effective, whereas metformin and SGLT2 inhibitors did not demonstrate significant protective effects compared with controls.

Conclusions: The number of patients with diabetes mellitus is increasing worldwide due to changes in lifestyle and social environment. Diabetic patients are more likely to develop hearing loss (diabetes-related hearing loss), which often occurs from a relatively young age and significantly reduces quality of life (QOL). To clarify the mechanisms and effective preventive strategies of diabetic hearing loss, we investigated auditory function, cochlear histopathology, and preventive interventions using a type 2 diabetes mouse model.

SU116. Effects of Cholesterol Modulation on Cisplatin-Induced Hearing Loss

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Background: Cisplatin is a widely used and effective anticancer drug. However, it causes permanent sensorineural hearing loss in over 50% of treated patients. There are no FDA-approved therapies to prevent cisplatin-induced hearing loss (CIHL) in adults, highlighting a critical unmet clinical need. Previous studies suggest that statins, commonly prescribed cholesterol-lowering drugs, are associated with reduced incidence and severity of cisplatin-induced hearing loss. The mechanisms underlying the protective effect of statins are unclear; however, the hallmark activity of statins is inhibition of HMG Co-A reductase, resulting in reduced serum cholesterol. Thus, to begin to address the mechanisms underlying statin-induced protection, we asked whether a genetic model with reduced serum cholesterol is also protective.

Methods: Here we examine whether reduced plasma cholesterol confers protection against CIHL independent of statin treatment. We utilized mice lacking serine protease proprotein convertase subtilisin/kexin type 9 (Pcsk9 knockout mice) as a genetic model of reduced plasma cholesterol.

Results: We find that Pcsk9 KO mice have reduced cholesterol compared to their WT littermates. Pcsk9 KO mice were significantly protected against cisplatin-induced hearing loss, as reflected by significantly lower ABR and DPOAE threshold shifts relative to WT. Histological analyses confirmed preservation of cochlear outer hair cells (OHCs) in Pcsk9 KO mice treated with cisplatin, whereas WT mice showed significant OHC loss in the high-frequency cochlear regions. Finally, hearing loss positively correlated with baseline plasma cholesterol levels.

Conclusions: Together our data demonstrate that systemic cholesterol reduction provides significant protection against CIHL, and they suggest that the protective effect of statins against CIHL is, in part, mediated by cholesterol reduction.

SU117. Connexin30-Deficient Mice Increase Susceptibility to Noise via Redox and Lactate Imbalances

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Background: Noise significantly contributes to one-third of the global burden of hearing loss. The intricate interplay of genetic and environmental factors impacts various molecular and cellular processes that lead to noise-induced hearing loss (NIHL). Defective connexin 26 (Cx26) and connexin 30 (Cx30), encoded by Gjb2/Cx26 and Gjb6/Cx30, respectively, are prevalent causes of hereditary deafness. However, the role of Cx30 in the pathogenesis of NIHL remains unclear.

Methods: Cx30-deficient mouse models related to NIHL were established and subjected to audiological follow-up using auditory brainstem response (ABR) measurements. Cochlear morphology was analyzed via immunofluorescence, transmission electron microscopy, and toluidine blue staining. Changes in oxidative stress were observed and compared using dihydroethidium (DHE), 4-hydroxynonenal (4HNE), and western blot assays. Cochlear glucose transport and energy metabolism were assessed via 2-NBDG glucose uptake and ATP release assays. Metabolomic analysis was employed to investigate mechanisms underlying cochlear energy alterations.

Results: We observed that homozygous Cx30 knockout (Cx30 KO) mice exhibited poorer hearing recovery after noise exposure (97 dB mean sound pressure level for 2 h) and increased susceptibility to noise. In addition to the exacerbation of noise-induced damage to hair cells and synapses, Cx30 KO mice exposed to noise exhibited increased oxidative stress. The 2-NBDG assay showed a reduction in glucose levels associated with a decrease in gap junctions as well as a reduction in adenosine triphosphate release. Glucose metabolomics analysis further revealed that Cx30 KO mice had elevated lactate and NAD⁺ levels after noise exposure, thus worsening anaerobic oxidation from glycolysis.

Conclusions: Our study emphasizes that Cx30-deficient mice increase susceptibility to noise via redox and lactate imbalances in the cochlea.

SU118. The Cisplatin-Induced Hearing Loss Model in Rats: An In-Depth Characterization to Optimize Therapeutic Development

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Background: Cisplatin is a platinum-based chemotherapeutic agent widely used in the treatment of many cancers. However, its use is often associated with severe side effects including permanent hearing loss and cochlear inflammation. Despite its extensive use in clinical treatments, few protective strategies and no effective regenerative therapies are currently available. The development and characterization of experimental animal models are essential for understanding the mechanisms of cisplatin's ototoxicity and for developing effective treatments. This study aimed to optimize a reliable preclinical rodent model of Cisplatin-Induced Hearing Loss (CIHL) and to characterize the associated hearing impairment at functional, cellular, immunological and protein levels to efficiently develop and evaluate therapeutic compounds.

Methods: CIHL model was induced by administering a single intraperitoneal (IP) injection of cisplatin to rats (T0). Different doses of cisplatin were tested (8, 10 and 13 mg/kg). The ototoxic effects of cisplatin were evaluated and characterized using several methods: (1) Distortion Product Oto Acoustic Emission (DPOAE) and Auditory Brainstem Response (ABR) measures were conducted before and after cisplatin administration (T+3DAYS) to assess auditory function; (2) histological analyses were performed on cochleae sampled at T+3DAYS to assess hair cell (HC) loss and analyse the immune response induced by cisplatin exposure; (3) protein analyses were carried out on cochleae sampled at T+4HOURS using specific arrays to determine the signaling pathways activated by cisplatin injection.

Results: At T+3DAYS, DPOAE and ABR measures showed a dose-dependent alteration of auditory function at 10 and 13 mg/kg of cisplatin, indicating a progressive hearing loss. In contrast, a single dose of 8 mg/kg of cisplatin did not produce significant alterations in auditory function compared to controls. Immunostaining to quantify Inner and Outer Hair Cells (IHCs and OHCs) showed that the auditive deficits observed at 10 and 13 mg/kg were accompanied by a reduction in OHC density in a dose dependent manner. Histological analyses of the immune response were conducted on cochleae prepared either as flat surface or cross-sections. Ongoing analysis allowed the identification and quantification of macrophages (Iba1), T lymphocytes

(CD3) and B lymphocytes (PAX5) in the cochleae of rats exposed to cisplatin. Finally, protein analysis of cochlear tissue, targeting over 200 molecules, led to the identification and quantification of several cytokines, chemokines, growth factors and soluble receptors, most of which were classified as inflammatory and trophic factors.

Conclusions: Preclinical testing is a critical phase in drug development, underscoring the need to refine and expand tools to support in-depth characterization of in vivo models to improve the advancement of protective or regenerative therapies. In this study, 10 mg/kg of cisplatin lead to a relevant CIHL model. Its characterization from the functional to the molecular levels, including inflammatory component, yielded valuable data for the development of new therapeutic compounds.

SU119. Immune Cells and Hearing Disorders: Development of a Short In Vivo Model for Evaluating the Anti-Inflammatory Potential of Therapeutic Compounds

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Background: Following exposure to noise, ototoxic agents, or aging, immune cells, particularly macrophages, are recruited to the cochlea to contribute to tissue repair. However, their excessive activation can lead to chronic inflammation, resulting in cochlear damage and hearing loss. A better characterization of macrophage recruitment kinetics, localization and signaling pathways is essential for optimizing the therapeutic potential of anti-inflammatory compounds. This study aimed to establish a short and well characterized in vivo model of cochlear inflammation to efficiently evaluate anti-inflammatory compounds.

Methods: Cochlear inflammation was induced in mice by transtympanic (TT) injection of lipopolysaccharide (LPS) in both ears (T0). Different parameters were evaluated: the dose of LPS (5 and 10 µg/ ear) and the timepoint for histological analyses of the immune response (T+2DAYS, T+4DAYS, T+7DAYS). Auditory Brainstem Response (ABR) thresholds were measured at each timepoint to assess associated hearing impairments. Histological analyses were conducted on cochleae prepared either as flat surface or cross-sections, followed by immunolabelling to identify macrophages (Iba1), T lymphocytes (CD3) and B lymphocytes (PAX5). Immune cells quantification and qualitative assessment of their localization in the cochlea were performed to correlate with ABR outcomes.

Results: LPS injection induced a significant increase in the number of immune cells in the cochlea, as demonstrated in flat surface preparations and cross sections. Immunolabelling of macrophages, T and B lymphocytes revealed that the immune response was mainly formed of macrophages, detected in the spiral ligament and spiral ganglion neuron regions. Macrophage recruitment in the cochlea was transient and increased at T+2DAYS and T+4DAYS, but not at T+7DAYS, compared to controls. Interestingly, in mice injected with LPS, the number of macrophages correlated with both the dose of LPS and the increased ABR thresholds at T+2DAYS and T+4DAYS. At T+7DAYS both markers completely recovered.

Conclusions: In LPS cochlea, a huge recruitment of inflammatory cells was reported, thanks to histological tools, and correlated with transient hearing loss. These results validate the local use of LPS as a rapid and efficient method to induce cochlear inflammation, providing a relevant in vivo model to evaluate anti-inflammatory compounds in vivo and their effects on hearing. Immune response characterization is now being completed with protein analysis of cochleae and Complete Blood Count (CBC) tests to further characterize the inflammatory response in this model.

SU120. Can Oncomodulin Be Expressed in Macrophages Following Cochlear Damage?

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Background: Oncomodulin (OCM) is the most abundant calcium-binding protein in outer hair cells (OHCs). In mice, the absence of OCM leads to progressive hearing loss and degeneration of OHCs, indicating that OCM is essential for maintaining cochlear function. Research has shown that OCM is transiently expressed and secreted by macrophages and neutrophils in response to injuries in the eye and spinal cord. The released OCM promotes nerve regrowth and repair for retinal ganglion cells and dorsal root ganglia. However, it remains unclear whether OCM can also be expressed in macrophages and neutrophils following injuries in the cochlea, just as in damaged eye and spinal cord. In the current study, we aim to investigate OCM expression in macrophages after cochlear damage using a new *Ocm*-KO/*lacZ* reporter mouse model (*Ocm* *tm1b*), which replaces *Ocm* translation with the expression of β -galactosidase (β -gal).

Methods: We created an *Ocm*-KO mouse model (*Ocm* *tm1a*) based on the ‘KO-first, conditional-ready’ strategy. *Ocm* *tm1a* mice were then crossed with CMV-Cre mice, which can delete the neo cassette and exon 3 of the *Ocm* *tm1a* allele to generate a *lacZ*-tagged global KO allele (*Ocm* *tm1b*). To confirm the absence of OCM in *Ocm**tm1b*/*tm1b* mice, we collected cochleae from WT and *Ocm**tm1b*/*tm1b* mice for immunofluorescence (IF) and Western blot with an antibody for OCM. To validate β -gal protein expression in the OHCs of *Ocm**tm1b*/*tm1b* mice, we performed X-gal staining in the cochleae from WT and *Ocm**tm1b*/*tm1b* mice. Additionally, we performed IF with antibodies for β -gal and OHC marker Prestin. To investigate whether OCM is expressed in macrophages within damaged cochlea, we administered a single intravenous dose of 16 mg/kg cisplatin to *Ocm**tm1b*/*tm1b* mice. One and two days after injection, we collected cochleae to perform IF with antibodies for β -gal and macrophage marker IBA1.

Results: OCM protein expression was not detected in *Ocm**tm1b*/*tm1b* cochleae, but it was present in WT cochleae. Additionally, β -gal protein expression was observed in the OHCs of *Ocm**tm1b*/*tm1b* cochleae but not in those of WT cochleae. There was no detectable β -gal staining in macrophages labeled with anti-IBA1 in untreated *Ocm**tm1b*/*tm1b* cochleae. However, a few β -gal and IBA1 positive cells were found in the stria vascularis of the *Ocm**tm1b*/*tm1b* cochleae collected 1 day after cisplatin injection.

Conclusions: These results show that OCM can be transiently expressed in macrophages in damaged cochlea. Since cisplatin damages many tissues and cells, it remains unclear whether the β -gal and IBA1 positive cells originate from resident macrophages in the cochlea or circulating

monocytes from the blood due to cisplatin-induced damage in other tissues. To address this uncertainty, we will use noise exposure to induce tissue damage exclusively in the ear. (Work supported by the Knowles Leadership Fund and NIH R56DC020542 to JZ).

SU121. Spatial Organization of Cochlear and Vestibular Lymphatics in the Mouse Model

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Background: The lymphatic system circulates interstitial fluid and immune cells to return to the bloodstream, playing a critical role in tissue fluid homeostasis and the immune surveillance system. The system consists of lymphatic vessels (lymphatics) and lymph nodes. For many years, the nervous system has been considered devoid of a lymphatic vascular system. Recent discoveries of the lymphatic vascular systems draining the brain and cerebrospinal fluid have brought a paradigm shift. Lymphatics of some sensory systems, like the visual systems, have also been described. However, the lymphatic vascular system for the sensory organs of hearing (cochlea) and balance (vestibular system) has never been described. The current study is aimed at exploring the possibility of lymphatic vessels in the cochlea and vestibular systems.

Methods: 8-12-week-old mice were used to identify the cochlear and vestibular lymphatic vessels. Thy1+/YFP and PECAM1-EGFP mice on C57B6 background were used to differentiate lymphatics from SGNs and blood vessels, respectively. The cochlea and vestibule, fixed with 4% PFA, were decalcified with 0.12M EDTA and cryopreserved with increasing concentration (10%-30%) of sucrose. 30-100µm thick cochlear and vestibular sections and cochlear whole mounts were labeled with antibodies against lymphatic markers (PDPN, Lyve1, VEGFR3), anti-GFP (to amplify Prox1-EGFP signal), and Anti-myosin VIIa (hair cell marker) antibodies and imaged using a confocal microscope. Whole cochlea and vestibule samples labeled with markers of lymphatics were cleared using the CUBIC tissue clearing protocol.

Results: A robust lymphatic vascular network, labeled with markers of lymphatics, was observed in the cochlea and vestibular systems. Within the cochlea, lymphatics are observed in the spiral ganglia, stria vascularis, spiral ligament, Reissner's membrane, along the margins of the scala tympani and scala vestibuli, modiolus, and underneath the hair cells. PDPN+ lymphatics could be distinguished from Thy1+ SGN fibers and PECAM1+ blood vessels. We have observed the lymphatic vessels in different parts of the vestibular system, including the saccule, utricle, vestibule, and semicircular canals. Expectedly, both cochlear and vestibular lymphatics are adjacent to the cochlear blood vessels and located in strategic locations suitable for draining major sensory structures, and interstitial (perilymph and endolymph) fluids.

Conclusions: Our results suggest the presence of a cochlear and vestibular lymphatic network in adult mice. Further investigations using different lymphatic markers are necessary for anatomical and molecular characterization of cochlear lymphatics.

SU122. Role of the Cochlear ‘Immune Surveillance System’ in the Foreign Body Response Following Cochlear Implantation

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Background: The inflammatory foreign body response (FBR) following cochlear implantation (CI) has the potential to negatively impact CI outcomes, including increased electrode impedances, accelerated loss of residual acoustic hearing, reduced battery life, and, rarely, implant extrusion. Immune cells contribute to the FBR post-CI. Immune cells are trafficked to the tissue via blood vessels and return to the bloodstream via the lymphatic vessels, forming the ‘immune surveillance system’. This study aims to investigate the role of immune cell trafficking via cochlear blood vessels and the recently discovered cochlear lymphatic network in the FBR post-CI.

Methods: To investigate the immune cell trafficking through cochlear blood vessels and lymphatics, we used five mouse strains: CX3CR1+/GFP (reporter for mononuclear phagocytes/MNPs), CCR2+/RFP CX3CR1+/GFP (reporter for blood-derived/ hematogenous/ infiltrated/extravasated MNPs), Prox1tdTomato-CD11cGFP (lymphatic-dendritic cells dual reporter), Prox1tdTomato-CX3CR1+/GFP (lymphatic-mononuclear phagocyte dual reporter), and Prox1tdTomato-PECAM1GFP (lymphatic-blood vessel dual reporter). The left ears of 10-12-week-old mouse models were implanted with a standard non-functional CI. Following the euthanasia of mice at 28 days post-operatively, harvested cochleae fixed with 4% PFA were processed and cryosectioned at 30µm parallel to the mid-modiolar plane. Sections labeled with antibodies against appropriate markers were used to identify different cell types: CD45, CD3e, MHCII, anti-GFP, Lyve1, VEGFR3, and PDPN. Using IMARIS image analysis software, the density of MNPs, infiltrating immune cells, CX3CR1+MHCII+ (APC), CX3CR1-CD45+ (non-MNP immune cells), CX3CR1-CD45+ CD3e+ (T lymphocytes), and CD11cGFP+ (dendritic cells) was calculated. The volume of blood vessels (PECAM1+) and lymphatic vessels (Prox1tdTomato+, PDPN+VEGFR3+Lyve1+) was measured within the scala tympani of the base of the cochlea, and the density of the blood vessels and lymphatic vessels in the scala tympani of the cochlea was quantified. Intralymphatic immune cell density was quantified, creating masks for lymphatic vessels and the immune cells.

Results: Following CI, blood vessels proliferate into the cochlea, trafficking blood-derived immune cells into the cochlea. Cochlear implantation also leads to lymphatic vessel proliferation into the cochlea, trafficking immune cells. Recruitment of MHCII+APCs, dendritic cells, and T lymphocytes suggests an activation of the adaptive immune system.

Conclusions: Our data indicate that following cochlear implantation, vascular proliferation is associated with a two-way traffic of immune cells to (via blood vessels) and away (via lymphatics) from the cochlea. This supports the involvement of the cochlear ‘immune surveillance system’ in FBR post-CI.

SU123. Diagnostic Yield of Whole-Genome Sequencing in Hearing Impairment: A Five-Year Study in a Large German Cohort

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Background: Hereditary hearing loss is a genetically and clinically heterogeneous condition with variable age of onset, severity, and progression. Genetic testing has shifted from targeted panels and exome sequencing toward whole-genome sequencing (WGS), which enables more comprehensive variant detection and deeper insights into the genetic spectrum of hearing loss. This study aimed to retrospectively evaluate five years of whole-genome sequencing for hereditary hearing loss at a tertiary care center in Southwest Germany by assessing diagnostic yield, the spectrum of causative genes, and factors influencing the likelihood of a molecular diagnosis.

Methods: 383 index probands with suspected hereditary hearing loss were identified, who underwent WGS between April 2020 and July 2025. Phenotypic characterization was performed by reviewing the electronic medical records and the institutional audiometric database, collecting information on family history, age of onset, degree and laterality of hearing loss. These phenotypic variables were additionally complemented by genetically derived estimates of ethnicity and consanguinity. Logistic regression was applied to estimate the effects of predictors on diagnostic outcome, with significance levels adjusted for multiple testing.

Results: The age at genetic testing ranged from 0.2 to 84.6 years (mean $\pm$ SD: 33.8 ± 23.1 years). A postlingual onset of hearing loss was present in 53.0% of probands, while 31.3% presented with congenital and 14.9% with early-onset (pre- or perilingual) hearing loss. A positive family history of hearing loss was reported in about half of the probands (53.0%). Of the 383 probands analyzed, a genetic diagnosis was obtained in 106 cases (27.7%). Among the solved cases, autosomal-recessive inheritance predominated (63.2%), followed by autosomal-dominant inheritance (30.2%). The underlying variants were distributed across 37 genes, with a marked predominance of GJB2, which accounted for 35% of all solved cases. Six additional genes contributed 24.5% of diagnoses, including MYO6 (5.7%) and COCH, MYO15A, MYO7A, PTPRQ, and TMPRSS3 (all 3.8%). The remaining 40.5% of genetically diagnosed probands were linked to variants in 30 other genes. In logistic regression analysis, age at onset of hearing loss, positive family history, and degree of hearing loss emerged as significant predictors of diagnostic outcome. Pairwise contrasts of estimated marginal means further indicated higher predicted diagnostic rates in probands with a positive family history compared to those without, and in congenital or early-onset hearing loss compared to postlingual onset.

Conclusions: In this large German cohort, genetic testing for hereditary hearing loss revealed a broad spectrum of causative genes and provided an overview of the genetic landscape in the population, highlighting its clinical relevance. Certain clinical factors emerged as predictors of diagnostic outcome, emphasizing the value of detailed phenotyping for genetic diagnostics in hearing loss.

SU124. Inner Ear Malformations in Patients With Hearing Loss — Exploring the Genetic Architecture

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Background: Inner ear malformations (IEMs) can occur in isolation or as part of genetically determined syndromes. Enlarged vestibular aqueduct (EVA) is the most frequently observed IEM, often accompanied by incomplete partition type 2 of the cochlea and a dilated vestibule. SLC26A4 is the major gene associated with IEMs, with 50% of EVA/IP2 patients carrying either two pathogenic SLC26A4 variants or one SLC26A4 pathogenic variant together with the CEVA haplotype. The genetic background of other IEMs is more diverse and remains poorly understood.

Methods: DNA was isolated from blood samples of 40 hearing loss (HL) patients with bilateral IEMs. Sequencing was performed using a custom multigene panel (237 or 263 genes).

Results: Genetic testing identified the cause of IEMs in 67.5% (27/40) of individuals. Among EVA/IP2 patients, six carried two pathogenic variants in SLC26A4, while another six had one SLC26A4 pathogenic variant together with the CEVA haplotype. The genetic cause still remains unknown in six EVA/IP2 patients. In patients with other IEMs, pathogenic variants were identified in POU3F4 as well as genes associated with CHARGE (CHD7), BOR (EYA1, SIX1), Waardenburg (SOX10), and Kabuki (KMT2D) syndromes. Additionally, a novel pathogenic variant in HOXA2 was identified in a single patient.

Conclusions: Mutations in SLC26A4 and the CEVA haplotype account for more than half of EVA/IP2 cases. Patients with other IEMs are frequently diagnosed with syndromic forms of HL. Further research is needed to identify pathogenic variants in noncoding regions of known HL genes or in novel genes.

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SU125. Immune Modulation of Zfp719 Dependent Hearing Loss

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Background: Hearing loss affects ~20% of the population, with sensorineural forms predominating. Causes include genetics, environmental factors, trauma, and aging. Adult-onset hearing loss not only impacts individual well-being but also carries substantial socioeconomic implications. Our research seeks to elucidate unexplained genetic underpinnings of adult-onset hearing loss, focusing on rare coding variants within established and undiscovered hearing loss genes. Taking advantage of a genome first approach in the Penn Medicine BioBank and functional studies in mice we identified ZNF175, and its mouse orthologue Zfp719, as a novel adult onset hearing loss gene. ZNF175/Zfp719 is a member of a large family of Krüppel-

associated box zinc-finger proteins that bind DNA and silence target gene expression, including transposable elements and host genes, through the formation of heterochromatin.

Methods: Genomic and bioinformatic analysis of snRNA-seq datasets, quantitative spatial transcriptomics, a flexible genetic system in mice were used to identify the gene targets of Zfp719 mediated heterochromatin silencing and determine the pathogenic consequences of their misregulation on calcium ion homeostasis, ribbon synapse integrity, hair cell viability, and innate immunity.

Results: Zfp719 mutant mice exhibited progressive synaptopathy, hair cell degeneration and hearing loss, with severity increasing in both an age- and gene dosage- dependent manner. Tissue-resident macrophages infiltrated the cochlea before overt signs of cellular damage and persisted as pathology advanced along the cochlear duct from base to apex. Selective deletion of Zfp719 in hair cells, but not in spiral ganglion neurons, recapitulated these cochlear defects, including macrophage activation. Strikingly, pharmacological depletion or genetic disruption of macrophages accelerated cochlear pathology in Zfp719 mutants, revealing a protective role in delaying both the onset and severity of hearing loss. Single-nucleus transcriptional profiling identified Gm15083, a long non-coding RNA, as one of the most upregulated genes in Zfp719 mutants. Gm15083 overlaps with Atp2b2 on chromosome 6 and is transcribed in antisense orientation, raising the possibility that derepression of Gm15083 interferes with Atp2b2 transcription. Atp2b2 encodes plasma membrane calcium transporting ATPase 2 (PMCA2), a crucial efflux pump required to maintain calcium homeostasis in outer hair cells. Consistent with this model, Atp2b2 decreased and Gm15083 increased in Zfp719 mutants outer hair cells. Moreover, Atp2b2 haploinsufficiency closely phenocopied the Zfp719 mutant phenotype, implicating disrupted calcium homeostasis as a primary driver of hearing loss. Ongoing experiments are testing whether loss of Gm15083 transcription restores Atp2b2 expression and auditory function in Zfp719 mutants.

Conclusions: Zfp719 is required in cochlear hair cells for normal auditory function, and its loss triggers gene dosage-dependent degenerative defects. Cochlear macrophages act protectively in this context. The concordant changes in Gm15083 and Atp2b2 expression indicate that disrupted calcium homeostasis is a principal consequence of Zfp719 deficiency. Given the multifactorial etiology of adult-onset hearing loss, our findings may help inform a broader understanding of the condition.

SU126. USH2A Variants as a Significant Genetic Factor an Mild to Moderate Hearing Loss

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Background: The most common genetic cause of mild to moderate hearing loss (HL) is STRC copy number variants (CNVs). Many patients with mild to moderate HL require further genetic testing due to the lack of molecular diagnosis. Literature indicates that, besides STRC, USH2A, MPZL2, and OTOG may also be responsible for mild to moderate HL. This study aimed to identify the genetic basis of isolated mild to moderate HL in patients without STRC CNVs.

Methods: The study included 47 patients with isolated mild to moderate HL without detected STRC CNVs or with one identified heterozygous STRC CNV. Genomic DNA was isolated from peripheral blood and NGS with a custom panel of HL-related genes (n=237 or 263) was performed. Selected variants were confirmed by Sanger sequencing.

Results: NGS revealed the genetic cause of HL in 51% (24/47) of patients. Among these, 25% (6/24) of causative variants were found in USH2A, linked to Usher syndrome. In 17% (4/24) of variants, mutations in OTOG were considered as the cause of HL. The remaining causative variants were detected in: ADGRV1, GATA3, MPZL2, OTOA, POU3F4, PTPRQ, REST, STRC, TBC1D24, and WFS1.

Conclusions: NGS revealed that, in addition to STRC, a significant proportion of patients with mild to moderate HL have causative variants in USH2A. Patients with USH2A pathogenic variants are likely to develop Usher syndrome type 2A, which includes both HL and retinitis pigmentosa. Identifying the genetic cause is crucial for providing personalized care from an early age.

Additional information: National Science Centre, Poland, 2020/37/N/NZ5/02800

SU127. Identification of Pathogenic Synonymous SLC26A4 Variants

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Background: Variants in SLC26A4 are a common cause of autosomal recessive hearing loss (HL) associated with an enlarged vestibular aqueduct (DFNB4/non-syndromic enlarged vestibular aqueduct (EVA)) and Pendred syndrome (HL and thyroid goiter). In ~15-30% of cases, biallelic variants SLC26A4 are not identified, suggesting the presence of an unidentified second pathogenic variant. We hypothesized that in some unresolved cases, the second variant is a splice-altering synonymous change.

Methods: A prospective study identified candidate variants in persons tested using the latest version of our comprehensive genetic testing platform (OtoSCOPE®). Variants were filtered and prioritized based on minor allele frequency, in silico predictions, family history, and clinical correlation.

We also performed a retrospective analysis of 199 persons with a single SLC26A4 pathogenic/likely pathogenic variant, a phenotype potentially consistent with SLC26A4-related HL, and no genetic diagnosis using the current version of the bioinformatics workflow, which assesses synonymous variants to identify synonymous variants of interest that were being previously filtered out.

The functional impact of candidate variants was assessed using minigene splicing assays. Patient DNA containing the relevant SLC26A4 exon and a portion of the flanking intronic regions was

subcloned into a pre-constructed pET01 Exontrap vector encoding 5' and 3' exons separated by a multiple cloning site. Vectors were transfected into COS-7 cells. RNA harvest was performed 36-48 hours post-transfection. Changes in splicing were assessed using gel electrophoresis and Sanger sequencing.

Results: From the prospective analysis of patients, we identified a 2-year-old male with congenital, moderate-to-severe bilateral sensorineural HL and bilateral EVA. He carries the novel synonymous variant, NM_000441.2:c.600G > A, p.(Gln200=) together with a previously reported pathogenic variant, NM_000441.2:c.1151A > G, p.(Glu384Gly). The minigene splicing assay indicated that the c.600G > A variant results in skipping of exon 5, generating a frameshift.

From the retrospective analysis, we identified a 7-year-old female with sensorineural HL diagnosed at the age of 5, EVA, and a brother with HL. She carries the rare synonymous variant, NM_000441.2:c.1206G > A, p.(Val402=) variant together with the pathogenic variant, NM_000441.2:c.2284G > T, p.(Glu762*). The minigene splicing assay indicated that the c.1206G > A creates a cryptic splice acceptor 58 bases into exon 10 and a resulting frameshift.

Conclusions: This work highlights the importance of evaluating persons for splice-altering synonymous variants to identify pathogenic variants in autosomal recessive conditions. However, there are still many undiagnosed cases of presumed SLC26A4-related HL, highlighting the need for other methods of detection to resolve the challenge of missing heritability.

SU128. Identification of a Novel Founder EYA4 Structural Variant in North America

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Background: Autosomal dominant nonsyndromic hearing loss (NSHL) is a genetically heterogeneous disorder. Copy-number and structural variants (SVs) in EYA4 are a rare cause of hearing loss at the DFNA10 locus. We aimed to determine the genetic etiology of hearing loss in a series of families with autosomal dominant NSHL.

Methods: Retrospective review of patients referred for targeted genomic enrichment and massively parallel sequencing using the OtoSCOPE® panel, which covers all exons and flanking intronic sequences of known genes associated with NSHL. Genetic results and available phenotypic data were reviewed by a multidisciplinary team to assess variant pathogenicity. Haplotypes were reconstructed using short tandem repeat analysis (STRP) and variants in EYA4 and surrounding regions.

Results: A novel pathogenic SV in EYA4 was detected in five North American probands of European descent with family history suggestive of autosomal dominant NSHL, which involves a 5.8kb deletion of the C-terminal exons 19 and 20, and insertion of a 76-bp inverted downstream sequence. Segregation analysis revealed co-segregation with the hearing loss phenotype in 2 affected relatives. This SV is the most frequently detected pathogenic variant in

EYA4 in the OtoSCOPE® cohort, accounting for 5 of 25 (20%) of DFNA10 diagnoses to date and 5 of 15 (33%) diagnoses in probands of European ancestry. STRP haplotype analysis demonstrated an 857 kB region surrounding EYA4 in linkage disequilibrium, shared among all probands and affected family members.

Conclusions: This finding expands the mutational spectrum of EYA4-associated deafness, with identification of a novel SV as the most common pathogenic DFNA10 variant in a large hearing loss cohort. The complexity of this SV, coupled with haplotype reconstruction, strongly suggests a founder effect rather than a recurrent event. These data highlight the importance of critical analysis of high-quality next-generation sequencing in the assessment of complex SVs.

SU129. Genetic Insights Into Sensorineural Hearing Loss: Identifying Variants in Hispanic Children

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Background: Sensorineural hearing loss (SNHL) affects approximately 1 to 3 per 1,000 live births, with over half of congenital cases attributed to genetic causes. While major strides have been made in understanding the genetic basis of SNHL particularly in European and Asian populations, Hispanic/Latino populations remain vastly underrepresented in genetic studies and testing, limiting equitable access to diagnostics and potential interventions.

Methods: To address this disparity, we enrolled Hispanic-admixed pediatric patients with confirmed SNHL and their family members through collaborations across four sites in three countries. Saliva samples were collected using Oragene DNA kits, and comprehensive clinical information — including family history, audiologic evaluations, surgical reports, and imaging — was reviewed. Genomic DNA was isolated from saliva and submitted to either exome sequencing (n=26) at the University of Washington Northwest Genomics Core or whole-genome sequencing (WGS; n=25) at the University of Colorado Genomics Core. The generated sequence data were analyzed using a custom pipeline incorporating ANNOVAR and gnomAD minor allele frequency (MAF) filters. Prioritized variants were further assessed using functional prediction tools for single nucleotide variants and indels. Copy number or structural variants were called

using multiple tools including Manta, Strelka, CNVkit and FreeBayes. Identified pathogenic and putatively functional variants were Sanger-sequenced in the proband and other family members.

Results: Preliminary analyses based on exome sequence data identified several pathogenic or likely pathogenic variants in known hearing loss genes, including population-specific alleles such as GJB2 c.19C > T (p.Gln7*) that was previously identified in Ecuador and GJB2 c.131G > A (p.Trp44*) in Guatemala. Early findings suggest that allelic spectra defined by genes, variants, and MAF differ across recruitment sites and in comparison, to non-Hispanic populations reported in the literature. Furthermore, to date our cohort shows a higher proportion of syndromic relative to nonsyndromic HL, a trend supported by the few existing reports on Hispanic populations.

Conclusions: These results underscore both the shared and unique genetic architecture of SNHL in Hispanic groups. Importantly, the diagnostic yield from this study supports the inclusion of genetic testing in hearing care protocols for Hispanic children, with implications for early intervention and eligibility for emerging gene therapies. Future directions include analyzing additional WGS datasets, expanding recruitment across diverse Hispanic regions, optimizing variant interpretation, and disseminating findings to guide equitable implementation of precision medicine in otolaryngology.

SU130. Dysregulation of Extracellular Matrix and Inner Ear Components Underlying the Hypoplastic Endotype of Ménière's Disease: Role of ADAMTS18

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Background: Meniere Disease (MD) is a chronic inner ear disorder characterized by fluctuating sensorineural hearing loss (SNHL), tinnitus, and episodic vertigo, with variable severity and progression. Its etiology remains unclear, with autoimmune, viral, allergic, vascular, developmental, and genetic factors under investigation. Previous studies of human temporal bone consistently identified abnormalities of the endolymphatic sac in MD, correlating with distinct clinical pictures. In ~30% of MD cases, the sac remains developmentally arrested or hypoplastic (MD-hp) with patients showing early-onset disease, male predominance, bilateral symptoms, temporal bone malformations, and familial clustering of MD or SNHL. These features point to a genetic cause for this endotype, and our aim was to define its underlying basis.

Methods: MD patients were recruited following diagnostic guidelines, and the MD-hp endotype was identified using a surrogate radiodiagnostic marker in CT. Whole-exome sequencing was performed from saliva-derived DNA. Enrichment of rare SNPs and InDels (AF < 0.05) with predicted high/moderate impact were analyzed through gene burden analysis against general population databases. Structural variants were also called and prioritized by high constraint or pathogenic prediction. Functional analysis was performed with candidate genes revealing

implicated biological pathways. Candidate gene expression was further evaluated by immunohistochemistry in human and mouse temporal bone across different development stages.

Results: Our final cohort consisted of 42 MD-hp individuals. Candidate genes included 94 genes enriched in potentially causal variants. Functional enrichment analysis revealed biological pathways related to extracellular matrix formation and maintenance, including bone matrix development and non-syndromic hearing impairment. Among these, ADAMTS18 emerged as the strongest candidate with 35.7% of MD-hp patients carrying 15 distinct variants in this gene. Immunolabeling confirmed ADAMTS18 expression in human and mouse temporal bone during embryonic and early postnatal stages, both surrounding sensory epithelia and within the maturing cartilage destined to form the otic capsule.

Conclusions: Our findings support a strong genetic contribution to the MD-hp endotype, with an accumulation of variants in genes involved in inner ear structures and in the ossification of the surrounding temporal bone. In particular, ADAMTS18 harbors variants predominantly in MD-hp patients, and its disruption may underlie abnormal development of these structures during embryonic and early postnatal stages. Genetic delineation of this endotype provides new insight into disease mechanisms, supports stratification of MD patients into biologically meaningful subgroups, and may ultimately guide precision approaches for diagnosis and therapy.

SU131. Uncovering the Genetic Relationship Between Dementia and Age-Related Hearing Loss

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Background: Hearing loss is an important modifiable midlife risk factor for dementia. Recent studies have investigated the genetic relationship between dementia and age-related hearing loss (ARHL) with conflicting results. These analyses have conveniently treated ARHL and dementia as monolithic conditions, when in reality both encompass distinct pathophysiological processes. We have previously shown that genes linked to frontotemporal dementia may play a role in the metabolic subtype of ARHL (MHL), but not other ARHL subtypes. Other emerging evidence also suggests that genetic risk for hearing loss associates with specific brain structural changes, though these findings are typically based on broad hearing phenotypes. Therefore, we sought to employ a comprehensive phenotyping strategy using audiological measures and brain magnetic resonance imaging (MRI) data to examine whether genetic variants that we have found to be associated with MHL also affect brain structure, potentially providing mechanistic insight into the genetic relationship between ARHL and dementia.

Methods: Through the Canadian Longitudinal Study on Aging (CLSA), we have access to high-quality genotype and audiological data for 17,531 older adults. Estimates of MHL were calculated from audiograms of European-descent participants and subjected to genome-wide association study (GWAS) analyses to identify genetic variants associated with the MHL subtype. MRI GWAS data were previously generated by the UK Biobank (n=33,000) and accessed online. A phenome-wide association study (PheWAS) was performed to identify brain MRI traits that shared risk loci with MHL. Genetic colocalization was then performed to confirm that the same causal genetic variants were underlying these shared signals.

Results: PheWAS identified 7 MRI phenotypes with the strongest evidence of association ($P < 2 \times 10^{-5}$) with MHL risk variants. Colocalization analysis of these prioritized MRI-MHL-variant triplets identified strong evidence (92.8% posterior probability) for a shared genetic variant (rs4413512) in ARHGEF28 underlying both MHL and white matter microstructure in the retrolenticular segment of the internal capsule, a white matter pathway transmitting sensory information. This finding suggests that genetic variants associated with MHL may also affect brain white matter integrity, though the functional significance requires further investigation.

Conclusions: This exploratory study suggests (1) the utility of highly specific, objective phenotyping in genetic analyses of complex traits, and (2) preliminary evidence for shared genetic architecture between ARHL and brain white matter. The colocalization of an intronic variant in ARHGEF28 with white matter microstructure measures in the internal capsule may reflect the gene's dual role in regulating both cytoskeletal organization and RNA metabolism, two processes essential for maintaining axonal integrity and myelination. Looking ahead, future research expanding to additional dementia endophenotypes and ARHL subtypes will help clarify the role of hearing loss as a modifiable midlife risk factor for dementia, guiding both clinical decision-making and research on the hearing-dementia association.

SU132. Optimizing Genetic Testing Referrals for Pediatric Hearing Loss

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Background: Although the benefits of genetic testing in pediatric hearing loss are well established, referral rates for genetic testing remain low within otology and audiology practices. This study examined referral rates, referral pathways, and appointment completion for genetic evaluation in an ethnically diverse pediatric cohort before and after implementation of a quality improvement (QI) protocol.

Methods: In phase 1 (Jan-Aug 2023), chart reviews were conducted for 86 patients with hearing loss to determine if referrals to genetics were made. Data collected included demographics, referral modality (progress note documentation, routed note to genetics, or direct referral order) and genetics appointment status. In Phase 2 (Sep-Dec 2023), a standardized referral protocol was implemented requiring all newly diagnosed patients to be referred through one of three pathways (visit summary note, routed note, direct referral order). Providers received a brief training and reminder cards. Chart reviews were then conducted on 29 additional patients.

Results: In phase 1, 27.9% had a referral documented. Referrals were made by otologists (15.1%), audiologists (7%), or both (5.8%). A routed note occurred in 4.7% of cases, and a direct referral order in 15.1%. Among referred patients, 54.2% did not schedule an appointment, 20.8% had pending appointments, and 25% completed an appointment.

In phase 2, referral documentation increased to 55.2% of patients. Referrals were made by audiologists (31%), otologists (6.9%), or both (17.2%). A referral order was placed in 41.4% of patients, and 31% had a routed note. Appointment completion improved to 62.5% compared to 25% in Phase 1.

Conclusions: Genetic testing referrals remain underutilized but improved after implementing a standardized protocol. These findings emphasize the need to identify barriers in referral

pathways and provide ongoing provider training. Quality improvement projects offer a promising approach to optimize genetic evaluations and enhance precision care for pediatric hearing loss patients.

SU133. Estradiol Regulation of Cochlear Gene Expression in Female Mice

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Background: Sex is a biological factor that influences auditory function and disease susceptibility. Our recent study showed that sex affects cochlear gene expression, but the mechanisms regulating this sex-dependent expression remain unclear. Estrogen, a major female hormone, is known to modulate gene expression in many non-cochlear organs and tissues. This study aimed to investigate the effects of estradiol treatment on cochlear gene expression and to assess the functional implications of estrogen-responsive genes in the cochlea.

Methods: Young, healthy female CBA/CaJ mice were used. To eliminate gonadal hormone production, the mice underwent ovariectomy (OVX). OVX mice were then injected daily with 17 β -estradiol at a dose of 3 μ g dissolved in sesame oil for three consecutive days, while control OVX mice received equivalent volumes of sesame oil alone. At the end of treatment, mice were sacrificed and their cochleae were collected. Bulk RNA-sequencing was performed to compare cochlear transcriptomes between estradiol-treated and control mice. Differentially expressed genes (DEGs) were identified, and their functional associations were analyzed using multiple bioinformatics tools.

Results: Using the criteria of adjusted p-value < 0.05 and log2 fold change (FC) > 0.2, we identified 366 DEGs (165 upregulated and 201 downregulated). Of these, 98.4% were protein-coding genes, while the remainder were non-coding RNAs and pseudogenes. Most DEGs exhibited only mild expression changes with the log2 FC < 1. The five most upregulated genes with log2 FC > 1 were *Prl*, *Mgl2*, *Lars2*, *Siglec1*, and *Clec10a*. The three most downregulated genes were *Hspa1a*, *Kcnj11*, and *Hspa1b*. While these genes have diverse functions, many are involved in immune and stress responses. Gene Set Enrichment Analysis (GSEA) revealed distinct functional signatures for up- and downregulated genes. Among the upregulated genes, oxidative phosphorylation and fatty acid metabolism showed the highest normalized enrichment scores with adjusted p-value < 0.05. In contrast, downregulated genes were enriched for TGF- β (transforming growth factor β) signaling. This pathway regulates multiple processes, including development, immune function, and tissue repair. Additional biological processes and cell signaling identified through the Database for Annotation, Visualization, and Integrated Discovery (DAVID) included Wnt signaling, stress responses, transcriptional regulation, and biological rhythms.

Conclusions: Our study demonstrates that estrogen modulates the expression of cochlear genes with diverse functions, particularly those involved in energy metabolism and stress regulation. These findings enhance our understanding of sex-related differences in cochlear homeostasis and susceptibility to auditory disease and dysfunction.

SU134. Cellular and Transcriptional Heterogeneity Due to NF2 Deficiency: Insights From Human iPSCS

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Background: Loss-of-function mutations in NF2 give rise to NF2-related schwannomatosis (NF2-SWN), a rare autosomal dominant disorder characterized by the development of benign tumors of the nervous system, most notably bilateral vestibular schwannomas. Clinically, NF2-associated vestibular schwannomas (VS) present with a spectrum of symptoms including sensorineural hearing loss (SNHL), tinnitus, vertigo, and balance dysfunction.

Methods: Herein, we employed sex- and age-matched human iPSC lines, including CRISPR/Cas9-mediated isogenic NF2^{-/-} iPSC derived from healthy controls, as well as iPSC obtained from a patient with NF2 VS. To further delineate the effects of NF2 loss, we implemented a Tet-On NF2 inducible system in NF2-deficient cells to establish transcriptomic and phenotypic contrast between NF2 loss- and gain-of-function state. Through comprehensive transcriptome analysis, we traced molecular footprints and phenotypic features altered by NF2 deficiency in SCP state.

Results: Multimodal comprehensive transcriptome analysis revealed genes that are perturbed and directly involved in cellular heterogeneity due to biallelic loss of NF2. In addition, we consistently observed elevated expression of collagen-containing ECM genes and hyperactivation of PI3K/AKT pathways that are critically involved in cell survival, proliferation, and apoptosis. Thus, we investigated the cytotoxicity and evaluated the synergistic potential of the PI3K/AKT pathway inhibitors HCQ and QC in NF2-deficient SCP lines.

Conclusions: Our findings offer new insights into tumorigenesis and cellular heterogeneity in NF2-associated VS and highlight the value of patient-derived iPSCs and their derivatives as robust in vitro platforms for drug screening and therapeutic development. Ultimately, by repurposing FDA-approved drugs, HCQ and QC, we propose a more expeditious and safer approach to developing therapeutic strategies for NF2-associated VS.

SU135. The Role of a Conserved Extracellular Loop in TMC1 Gating

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Background: The transmembrane channel-like (TMC) family is an ancient group of proteins conserved across eukaryotes. During evolution, the mechanosensory channels TMC1 and TMC2 first appeared in the vertebrate lineage; they are expressed perhaps exclusively in vertebrate hair cells and seem to be highly specialized to mediate hearing and balance. In mammals, TMC1 appears to have undergone further specialization to function in its primary role in adult auditory transduction. Yet the molecular mechanism by which force opens TMC1 channels is not well understood. Here, we investigate the role of a conserved extracellular loop between transmembrane helices 1 and 2 (the 1-2 loop) that may contribute to channel gating.

Methods: We first used evolutionary analysis to find residues in TMC1 that were positively selected for and may underly its functional adaptations. We then used structural modeling to predict how these regions may affect overall structure and transduction complex binding partners. To assess functional consequences, we then selectively mutated individual amino acids in TMC1, constructed AAV vectors carrying the mutant sequence, injected AAVs into the cochleas of

Tmc1/2 null mice at P1, and recorded transduction currents from cochlear hair cells in vitro at P8-15.

Results: Evolutionary analyses revealed residues in the TMC1 1-2 loop that were positively selected in mammals. Structural modeling suggested that this loop arches over the extracellular side of the pore, approaching both the auxiliary subunit TM1E and the pore-lining helices TM4 and TM6. Mutating two conserved aromatic residues shifted channel activation towards the closed state without affecting slope, suggesting that these residues participate in biasing the channel to be open part of the time at rest.

Conclusions: A combination of evolutionary probing, structure analysis, and electrophysiology suggest the 1-2 loop has a specialized role in mammalian hearing. Targeted mutagenesis influences channel activation and in following, this loop contains several residues linked to hereditary deafness. Integration of evolutionary, structural and physiological studies provides a holistic platform for investigating residues of functional importance in sensory transduction channels.

SU136. Estradiol Increases the Vulnerability to Hidden Hearing Loss in Female Mice Lacking GluA3 Ampar Subunits

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Background: The GluA3 AMPAR subunit is essential for cochlear ribbon synapse integrity. We previously showed that female GluA3-knockout (GluA3KO) mice develop an early-onset hidden hearing loss (synaptopathy) when raised in ambient sound levels, showing reduced ABR wave-1 amplitude, significant synapse loss and afferent terminals swelling by 5-weeks of age (Pal et al., 2025). This phenotype was absent in age-matched male GluA3KO mice (Rutherford et al., 2023). However, both female and male GluA3KO mice had higher GluA4:GluA2 ratio suggesting an increased in Ca⁺⁺-permeable AMPARs (cp-AMPA) at cochlear-ribbon synapses. This sex specific vulnerability suggests a hormone mediated mechanism. Among gonad-sex hormones, estradiol is thought to be otoprotective. However, it can also potentiate high-conductance synaptic cp-AMPA in the female brain (Jain and Woolley, 2023). We therefore hypothesized that in the absence of GluA3, estradiol could increase the hearing vulnerability to ambient sound, particularly affecting females.

Methods: To test the role of gonad sex hormones in hearing vulnerability, we performed bilateral gonadectomies (ovariectomy in females and orchidectomy in males) or sham surgery in female and male GluA3KO mice at postnatal day 22 (P22). Naïve wild-type (WT) female and male mice served as additional controls. We conducted a longitudinal assessment of cochlear function by measuring ABR clicks and pure-tones thresholds, and wave-1 amplitude and latency

in all groups at P20, P35, P65, and P90. Blood collection was performed at P90 to measure sex hormones levels. The structure of cochlear-ribbon synapses will be analyzed ultrastructurally.

Results: Only sham-operated female GluA3KO mice showed reduced wave-1 amplitude at P35 compared to WT controls, confirming early-onset of hidden hearing loss. Interestingly, ovariectomized female GluA3KO mice showed wave-1 amplitudes comparable to WT mice at P35. This protection persisted through P90, though wave-1 amplitude in ovariectomized GluA3KO females remained intermediate between WT and sham KO groups. In contrast, P35 male GluA3KO mice showed no hearing deficits, and orchidectomy had no effect.

Conclusions: Our results show a potential effect of estradiol on AMPARs at inner hair cell-ribbon synapses only in females. In addition, we show that estradiol increases the vulnerability of the female GluA3KO mice to hidden hearing loss.

SU137. Novel Stella Platform Reveals Single-Molecule Dynamics of MYO7A-Driven Active Cargo Transport in Live Hair Cell Stereocilia

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Background: Unconventional myosins are motor proteins that function on the actin cytoskeleton. Genetic variants of four unconventional myosin genes, MYO3A, MYO6, MYO7A, and MYO15A, have been associated with human hereditary hearing loss. In particular, MYO7A is presumed to transport components of the mechanoelectrical transduction (MET) machinery as their “cargo” and anchor them to the actin core of stereocilia. However, the molecular mechanisms underlying myosin-driven active cargo transport in stereocilia are only partially understood. For example, it remains unclear whether MYO7A and other myosins function as a monomer like class-I myosins, a dimer like class-V myosins, or a higher-order oligomer like non-muscle class-II myosins. To fill this knowledge gap, we established a novel single-molecule imaging platform, STELLA (Single-Molecule Tracking and Enhanced Localization in Live Aural specimens), and investigated how MYO7A molecules traffic within stereocilia of live murine hair cells (Miyoshi et al., *Nature Communications*, 2025).

Methods: STELLA is currently built on a diSPIM light-sheet microscope (STELLA-diSPIM). Utricles and saccules were harvested from C57BL/6J mouse neonates at postnatal day 2–5 and cultured on a collagen matrix. MYO7A fragments fused with an N-terminal HaloTag were expressed in hair cells via Helios® gene gun transfection. HaloTag was fluorescently labeled with JFX554-ligands, which are brighter and more stable than fluorescent proteins, and visualized with a 561-nm laser light sheet. MYO7A motor activity was activated by several approaches, including chemical dimerization using the FKBP-AP20187 system, missense mutations destabilizing its autoinhibitory conformation, and tethering to the plasma membrane or actin filaments via the FKBP-FRB-Rapalog system.

Results: Under STELLA-diSPIM, MYO7A exhibited processive movement toward stereocilia tips when (1) chemically dimerized and (2) constitutively activated by missense mutations

disrupting its tail-mediated autoinhibitory mechanism. Velocity was 101 ± 53 nm/s ($n = 42$, mean $\pm$ SD) for dimers and 73 ± 47 nm/s ($n = 18$) for activated mutants, which were similar by Student's t-test. Wild-type MYO7A did not show directional motility at a detectable frequency. These results suggest that MYO7A traffics as a dimer within stereocilia when released from the autoinhibitory conformation. Cargo proteins of MYO7A, particularly SANS and harmonin (USH1C), may mediate this activation. MYO7A appears to exhibit stepwise motility when its tail is tethered to actin filaments via harmonin b fragments, which may represent how tip links adjust position and tension in mature stereocilia.

Conclusions: Through STELLA microscopy, we established real-time dynamics analysis of protein molecules within live hair cells. Such methods will remain essential tools in hearing research, as the intracellular environment of inner ear hair cells is not easily reconstituted. STELLA can also be used to functionally evaluate and reclassify genetic variants of uncertain significance (VUS), particularly to identify those with hidden pathogenicity that may mislead clinical decision-making.

SU138. FOXO3 Protects Hearing in Mice by Maintaining Outer Hair Cell Mitochondrial Function

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Background: Noise induced hearing loss (NIHL) affects around 40 million adults in the United States, but little is known about genetic susceptibility to NIHL. FOXO3 is a conserved transcription factor with some genetic variants that are strongly linked to human longevity. We have previously found that homozygous Foxo3 knockout mice (Foxo3-KO) are more susceptible to noise damage than their wild-type littermates. Among other issues, outer hair cells (OHCs) in Foxo3-KO mice appear primed to die in response to a mild noise exposure. Here we compare the transcriptome of undamaged OHCs from wild-type and Foxo3-KO adult mice, in order to identify some of the mechanisms for this damage susceptibility.

Methods: A mouse line was created that harbored both the inducible CRE recombinase under control of the Slc26a5 (Prestin) promoter and the HA-tagged RPL22 (Ribotag) protein, which is incorporated into translating polyribosomes. These mice were bred to the Foxo3-KO line to obtain mice with one allele of Slc26a5-iCRE, one or two alleles of RPL22(HA), and either two wild-type or two knockout alleles of Foxo3. Mice were injected with tamoxifen to enable expression of RPL22(HA) in outer hair cells, which was validated by immunostaining. Immunoprecipitation was used to enrich for HA+ ribosomes from both wild-type and Foxo3-KO tissues. RNA samples were reverse-transcribed into cDNA and sequenced. Sequencing validation was performed with qPCR. Gene ontology terms were identified using R Studio. Adult OHC mitochondria were assessed using immunofluorescence, confocal microscopy, and blinded analysis in Imaris. OHCs in live preparations of organ of Corti from both genotypes were visualized with MitoTracker Green. Mitochondrial membrane potential was assessed with tetramethylrhodamine, calcium levels with rhodamine-2 AM, and superoxide levels with MitoSox.

Results: OHC expression of HA-tagged RPL22 was validated in in tamoxifen-injected littermates. Selected differentially expressed genes (DEGs) were validated by qPCR. Gene ontology analysis suggested that many terms associated with energy production differed between the genotypes. In particular, wild-type mice expressed higher levels of genes associated with mitochondrial maintenance, including the FOXO3 target Pink1. Morphologically, individual adult Foxo3-KO OHCs displayed equal volumes of mitochondria overall compared to OHCs in wild-types. However, individual mitochondria within Foxo3-KO OHCs were significantly larger, suggesting changes in mitochondrial dynamics. The live OHC mitochondrial analyses are ongoing, and results will be presented.

Conclusions: Mitochondrial dysfunction has previously been associated with hearing loss from noise, with some reports indicating that it may also play a role in NIHL. These findings suggest that FOXO3 promotes OHC survival after noise exposure in part through maintaining mitochondrial integrity and/or function. Understanding how mitochondria are involved in OHC resilience could lead to future interventions that help protect hearing from noise damage.

SU139. The Role of O-GlcNAcylation in the Inner Ear

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Background: Post-translational modifications are essential for fine-tuning protein function by altering their activity, localization, and interactions with other molecules. O-GlcNAcylation (O-GlcNAc) is a posttranslational modification present across various tissues. This modification is dynamically regulated by only two key enzymes: O-GlcNAc transferase (OGT), which adds the N-acetylglucosamine moiety, and O-GlcNAcase (OGA), which removes it, making it an attractive target for pharmacological manipulation. However, its role in inner ear physiology remains largely unexplored.

Methods: Here we investigated the role of O-GlcNAcylation in the inner ear, using electrophysiological patch-clamp measurements and immunohistochemical stainings in inner hair cells and spiral ganglion neurons in acutely isolated cochlear tissue from mice.

Results: In this study, we demonstrate for the first time the presence of O-GlcNAcylation in multiple inner ear cell types, including auditory sensory epithelial cells. Pharmacological modulation of OGT and OGA activity alters O-GlcNAcylation levels in these cells, indicating that this modification is active and regulated in the inner ear. Moreover, patch-clamp recording after the inhibition of OGT and OGA reveals alterations in voltage-gated K⁺, and Ca²⁺ channels, in inner hair cells and spiral ganglion neurons.

Conclusions: These findings suggest a potential regulatory role for O-GlcNAcylation in inner ear function and open new avenues for investigating its contribution to auditory physiology and pathology.

SU140. Further Characterization (Structural, Quantitative and Multi-Omic) of Inner Ear Mitochondria

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Background: In this study, we quantified multiple aspects of mitochondrial structure in the vestibular inner ear. Our main hypothesis in this research is that mitochondria are structurally heterogeneous, depending on which hair cell (HC) type is under consideration and the specific locations of mitochondria in relation to other hair cell organelles. One such structural relationship that we have reported on previously is the polarization of mitochondrial crista junctions (CJs), openings of the internal mitochondrial membrane into the intermembrane space that is likely responsible for transport of molecules from the mitochondrial matrix out to the cytoplasm.

Methods: Inner ear vestibular tissue obtained from Long-Evans rats under an approved UIC IACUC procedure was used for electron microscope tomography. Data were processed with the IMOD (v.3.13.6) software package (Kremer et al., 1996) and serial tomograms were joined using the etomo subroutine (IMOD). Manual segmentation was done with the 3dmod subroutine (IMOD) to obtain final models. Reconstructions were visualized using 3dmod (IMOD), including its SLICER option, to track relevant features in 3 dimensions. Several IMOD drawing tools (Sculpt, Join, Warp, Interpolator) and a proximity analysis tool (Mtk) with a randomization feature, were also used. Proteomic and genomic analyses are also being performed on the 3 different size classes.

Results: Quantitative analyses were performed to test our hypothesis, including total mitochondrial counts in both types of vestibular HCs (compared to cochlear HCs), efferent boutons, and afferent calyces, counts of CJs on either side of a mitochondrion relative to other cellular organelles, analyzing polarization ratios vs distances, bioenergetic calculations of ATP production, and an Mtk proximity analysis test, using CJ randomization as a control. Results support our hypothesis of structural heterogeneity of mitochondria, as CJs are polarized toward specific organelles within the cell and appear to be non-randomly distributed. Finally, vestibular HCs have < half the number of mitochondria compared to cochlear HCs, although some are larger than cochlear mitochondria.

Conclusions: These results have functional implications for the various structural differences. CJ polarization toward organelles that use energy can transport ATP more efficiently. For example, the cuticular plate is an organelle that functions to return stereocilia rootlets back to their original position. Our results show a non-random orientation of mitochondrial crista junctions toward the cuticular plate. These structural differences may provide key information to better understand their function and to address mitochondrial deafness and dizziness disorders.

SU141. MYO3A and MYO3B Regulate Stereocilia Tip Widening via Their Kinase and Motor Domains

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Background: The formation of stereocilia depends on tightly regulated actin dynamics and the targeted delivery of actin modulating proteins by non muscle myosins. Class III myosins, MYO3A and MYO3B, localize the actin binding proteins ESPN1 and ESPNL to stereocilia tips and contribute to stereocilia widening. Although mutations in Myo3a cause several hereditary hearing disorders, including DFNB30, the underlying pathogenic mechanisms remain poorly

understood. Both MYO3A and MYO3B possess an N terminal kinase domain that phosphorylates the motor region, thereby attenuating motor activity. In this study we dissect the distinct and overlapping contributions of MYO3A and MYO3B to hair bundle development.

Methods: We generated a series of mouse models: a Myo3a knockout, a Myo3a / Espn1 double knockout (to test whether MYO3B requires ESPN1 for tip localization), and a Myo3a knockout combined with a Myo3b allele bearing a four amino acid deletion in the N terminal kinase domain. Protein localization and hair bundle architecture were examined using high resolution Airyscan microscopy and expansion microscopy.

Results: Loss of MYO3A produced markedly elongated stereocilia throughout the cochlea, establishing its role in limiting stereociliary elongation. Although MYO3B tip localization increased in the absence of MYO3A, it offered only modest compensation; MYO3B failed to control short actin filament levels at stereocilia tips or to transport ESPN1 effectively. Strikingly, simultaneous deletion of MYO3A and ESPN1 yielded a distinct phenotype characterized by the loss of MYO3B from stereocilia tips and a sharp tapering of the tip region, indicating that MYO3 complexes specifically modulate actin incorporation along the upper shaft. Moreover, in the absence of MYO3A, the kinase deficient MYO3B mutant increased stereocilia length and altered distribution of MYO7A and EPS8L2, revealing an unexpected role for the kinase domain in regulating tip localized actin dynamics.

Conclusions: Our findings establish MYO3A as the dominant class III myosin in inner hair cells, essential for preserving normal stereociliary dimensions. The data illuminate how MYO3A mediated actin regulation shapes hair bundles and suggest mechanistic pathways by which MYO3A dysfunction contributes to DFNB30 associated deafness.

SU142. Functions of CCDC181 in Kinocilium Formation and Auditory Perception

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Background: Auditory hair cells exhibit a highly specialized ciliary architecture, consisting of a bundle of stereocilia that is responsible for the mechanotransduction and a single kinocilium that governs hair bundle polarity during development. In murine cochlear hair cells, the kinocilium appears during embryonic development alongside the formation of stereocilia bundles, and it degenerates by around postnatal day 12 as the stereocilia mature. Despite this well-defined developmental timeline, the molecular mechanisms governing kinocilium formation and subsequent degeneration remain largely unknown.

Methods: immunohistochemical staining, ABR, SEM, knockout mouse

Results: Through immunohistochemical staining, we found that CCDC181, a microtubule-binding protein, is abundantly expressed in the postnatal cochlear hair cells and specifically localized to the kinocilium. To investigate its function in kinocilium development, we generated a Ccdc181 knockout mouse. Hair cell morphology was characterized by using immunostaining and electron microscopy, and auditory function was then evaluated by measuring auditory brainstem responses.

Conclusions: Our preliminary data suggest that ablation of CCDC181 has minimal effects on kinocilium formation, hair cell polarity, or auditory function in mice.

SU143. Characterizing the Mechanosensitive Recruitment and Protein Partners of Hair Cell Repair Protein XIRP2

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Background: Hearing depends on the precise function of auditory hair cells, whose actin-based stereocilia deflect in response to sound to activate mechanotransduction channels. Hair cell death and damage to hair cell structures, such as stereocilia, can contribute to hearing loss. Therefore, understanding endogenous repair mechanisms is crucial for developing therapeutic targets for progressive hearing loss. Our lab previously found that noise exposure induces F-actin damage in stereocilia that is subsequently repaired, suggesting an endogenous repair process. A critical question remains: how do hair cells sense and repair F-actin damage, and how does this process prevent hearing loss? XIRP2, an actin-associated protein enriched in stereocilia, accumulates at damage sites, and Xirp2 knockout mice show impaired hair cell repair and progressive hearing loss. I hypothesize that XIRP2 is a key player in the hair cell actin repair process, working in concert with other stereocilia proteins that we aim to identify using proteomic approaches.

Methods: To investigate XIRP2's role in actin repair, we used two complementary approaches. First, we employed an in vitro model of actin damage to identify which domain of XIRP2 mediates its recruitment to F-actin lesions. We used laser photoablation to induce localized strain sites in stress fibers of fibroblast cells transfected with full-length or truncated XIRP2 constructs. Recruitment indices of each construct were compared to assess mechanosensitivity.

Second, to identify other proteins involved in hair cell repair, we developed a proximity labeling strategy in vivo. We generated a TurboID-XIRP2 knock-in mouse model, enabling biotinylation of proteins near XIRP2 in the context of F-actin damage. These proteins were then isolated and analyzed by mass spectrometry to define potential XIRP2 interactors.

Results: Our in vitro experiments indicate that XIRP2 is recruited to damaged actin in a force-dependent manner, and we have mapped the minimal mechanosensitive domain responsible for this recruitment. We also verified normal expression and localization of XIRP2 in the TurboID knock-in model. Mass spectrometry analyses identified candidate interactors that may participate in actin repair.

Conclusions: XIRP2's recruitment to actin damage requires its minimal mechanosensitive domain. Using our TurboID-XIRP2 knock-in strategy, we created and validated a new proximity labeling approach in auditory hair cells. This platform not only revealed candidate proteins involved in stereocilia repair but also establishes a broadly applicable method for studying protein interactions in the inner ear. By expanding the toolkit for proteomics in hair cells, this work provides new opportunities to uncover pathways that could be involved in progressive hearing loss.

SU144. Mechanoelectrical Transduction in Cnidarians Nematocytes and the Evolutionary Origin of Vertebrate Hair Cells

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Background: The conversion of mechanical stimuli to electrical signals is of fundamental importance in many physiological responses, including auditory and vestibular functions in vertebrates, and a host of sensory functions in invertebrates. Vertebrate inner ear hair cells contain a mechanoelectrical transduction (MET) complex located at the tips of stereocilia. Transmembrane Channel-like 1 and 2 (TMC-1/2) proteins in complex with have been suggested as the key mechanosensitive channel in this complex. Biochemical and structural characterization of the MET complex in vertebrate hair cells has been, and continues to be a major challenge.

Methods: We pursued morphological pharmacological, functional, bioinformatic and reverse genetic approaches to evaluate the evolutionary relationship between the cnidarian nematocyte and vertebrate hair cells.

Results: Here, we present new evidence pointing to a relationship between the vertebrate hair cell and the Cnidarian stereocilia-containing hair cells, which include nematocytes and other sensory ectodermal cells. Single cell differential transcriptomics together with targeted knock down of candidate mechanosensitive molecules, directly point to members of the TMC family of ion channels/scramblases as key participants in the generation of mechanically-evoked receptor potentials and the eversion of cnidocysts. Furthermore, dependence on extracellular Ca²⁺, pharmacological susceptibility to ototoxic aminoglycoside antibiotics and the spontaneous accumulation of the water-soluble styrene fluorophore FM1-43 also support an evolutionary relation between a putative MET complex in nematocytes and its equivalent in vertebrate hair cells.

Conclusions: Our computational, biochemical, and genetic studies corroborate a direct link to vertebrate hair cells and point to evolutionarily conserved mechanotransduction mechanisms.

SU145. The Role of PCDH15-CD3 in Stability of Tip Links in Mammalian Auditory Hair Cells

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Background: Sound detection is initiated by deflection of stereocilia, mechanosensitive projections at the apex of auditory hair cells. Stereocilia are arranged in rows of increasing heights and interconnected by extracellular tip links that convey forces to the mechano-electrical transduction (MET) channels. Tip links are formed by cadherin-23 (CDH23) and protocadherin-15 (PCDH15), at the upper and lower ends of a tip link, respectively. Mutations in genes encoding these proteins result in deafness. Hair cells express only one isoform of CDH23 but multiple isoforms of PCDH15, grouped into three classes (CD1, CD2, and CD3). Though both PCDH15-CD3 and PCDH15-CD2 are localized at the tips of stereocilia, only PCDH15-CD3 is

localized exclusively at the tips of transducing 2nd and 3rd row stereocilia within a hair bundle, suggesting the PCDH15-CD3 is the isoform class forming tips links. Yet, *Pcdh15-CD3*^{-/-} mice have normal hearing thresholds and overexpress PCDH15-CD2, indicating potential compensation by CD2. Further, disruption of tip links with Ca²⁺-free extracellular media results in the disappearance of PCDH15-CD3 immunolabeling, while PCDH15-CD2 immunolabeling is still present. We hypothesize that tip links of *Pcdh15-CD3*^{-/-} mice may have different stability and recovery after disruption.

Methods: We generated a novel mouse strain lacking only CD3 isoform of *Pcdh15* (*Pcdh15-CD3*^{-/-}). To study the role of CD3 isoform in tip link breakage and regeneration, we incubated organ of Corti explants (from *Pcdh15-CD3*^{-/-}, *Pcdh15-CD3*^{+/-}, and *Pcdh15-CD3*^{+/+} mice) in Ca²⁺-free medium (supplemented with 5 mM of BAPTA) for one minute to break the tip links and then followed tip link recovery in regular Ca²⁺-containing media at 0, 3, 6, 9, and 12 hours after breakage using high-resolution scanning electron microscopy (SEM). Control samples were incubated in regular Ca²⁺-containing HBSS (1.26 mM of Ca²⁺) and the tip links were visualized by SEM and counted at the same time points after incubation.

Results: 1) Shorter incubation in Ca²⁺-free medium supplemented with BAPTA (one minute instead of 15 minutes used previously) results in effective disruption of at least 80% of the tip links in wildtype inner hair cells but followed by a faster tip link recovery. 2) Tip links in *Pcdh15-CD3*^{-/-} hair cells recover more slowly after disruption. 3) Most strikingly, more tip links (up to 40%) are present in *Pcdh15-CD3*^{-/-} inner hair cells immediately after disruption with BAPTA and many of them appear to be shorter than normal.

Conclusions: So far, our data argues that the CD3 isoform is compensated by PCDH15-CD2 in *Pcdh15-CD3*^{-/-} hair cells, and these tip links are more resilient to disruption. The shorter length of these tip links also indicates that temporary homomeric PCDH15-PCDH15 links could be present due to overexpression of PCDH15-CD2.

SU146. Persistent Remodeling of Hair Cell Mitochondrial Networks in Response to Low Dose Cisplatin Exposure

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Background: Cisplatin is a widely used chemotherapeutic agent with broad efficacy. However, its clinical utility is limited by dose-dependent toxicities, including ototoxicity that can lead to permanent hearing loss.

While high-dose cisplatin-induced hair cell apoptosis has been well characterized, the cellular effects of clinically relevant, sub-lethal doses remain poorly understood. Notably, some patients experience delayed-onset, progressive hearing loss after treatment, suggesting that surviving hair cells may undergo lasting subcellular stress. Mitochondria have been identified as early and critical targets of cisplatin-induced death in hair cells (Lee, 2024), yet how mitochondrial structure and function respond to low dose, non-lethal exposure has not been fully explored.

In this study, we used a zebrafish model to investigate the effects of low dose cisplatin exposure on hair cell mitochondria. We assessed mitochondrial morphology, calcium dynamics, and membrane potential as indicators of early dysfunction, to better understand low dose cisplatin-induced hair cell injury and identify potential mechanisms of vulnerability and repair.

Methods: Transgenic zebrafish larvae expressing a mitochondrial calcium indicator in hair cells (Tg(myo6b:mitoGCaMP)) were exposed to (30 μ M, 100 μ M, 300 μ M, 1 mM) cisplatin for 2 hours, followed by (0, 6, 24, 48, 72) hour recovery. Larvae were then fixed in paraformaldehyde and imaged using super-resolution confocal microscopy. Mitochondrial morphology was quantified using 3D image analysis. Ongoing studies include live imaging of mitochondrial membrane potential with TMRE and time-lapse super-resolution microscopy to assess dynamic mitochondrial remodeling. Functional consequences are being evaluated using a microflume-based rheotaxis assay to detect subtle deficits in hair cell-mediated behavior.

Results: Low dose cisplatin exposure induced significant changes in mitochondrial morphology in neuromast hair cells, even in the absence of overt hair cell loss. Our data revealed time and dose dependent changes in morphology features (count, branch length, branch junctions, sphericity) associated with mitochondrial network complexity. Notably, we observed the formation of toroidal (“donut”) mitochondria, which increased in frequency with higher cisplatin doses and persisted through the recovery period. Our data also suggests mitochondrial calcium dysregulation and loss of mitochondrial membrane potential. These changes were dose-dependent and evolved throughout the recovery period, with distinct morphological and functional trajectories observed at different concentrations.

Conclusions: Our findings show even clinically relevant, low dose, cisplatin causes persistent disruption of hair cell mitochondrial morphology and functionality. The persistence of mitochondrial morphological abnormalities supports a model in which hair cells experience chronic subcellular stress, potentially priming them for later vulnerability to additional insults, such as noise or aging. These insights lay the groundwork for developing targeted strategies to preserve hearing in patients undergoing cisplatin chemotherapy.

SU147. Live Imaging of Presynaptic Ribbons in the Aging Mouse Cochlea

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Background: Ribbon synapses in inner hair cells (IHCs) mediate rapid and precise neurotransmission to spiral ganglion neurons (SGNs), enabling accurate auditory signaling. With advancing age, both humans and mice experience synaptic alterations that contribute to hearing impairment. However, the cellular mechanisms underlying age-related synaptic decline remain poorly defined. Direct observation of presynaptic ribbon behavior across the lifespan may clarify how synaptic architecture changes with aging.

Methods: We used RIBEYE-tagRFP mice to visualize presynaptic ribbons in cochlear explants at three age groups: 3–4 months, 6 months, and 17–18 months. Whole-mount preparations were imaged with a Nikon A1R confocal microscope equipped with a 60 $\times$ water immersion objective. Confocal z-stacks spanning the IHC region were acquired, and RIBEYE puncta were analyzed using Imaris for number, size, and motility parameters. Group comparisons were made across age cohorts to identify trends in ribbon morphology and dynamics.

Results: Age was associated with progressive changes in presynaptic ribbon architecture. The total number of RIBEYE puncta decreased from young adult (3–4 months) to middle-aged (6 months) and further into aged (17–18 months) cochleae. In parallel, the average puncta size increased with age, suggesting enlargement or aggregation of remaining ribbons. Despite these morphological changes, ribbon motility, as assessed by speed of movement, remained similar across all three age groups. These findings indicate that while the structural landscape of synapses is altered during aging, the intrinsic dynamic properties of ribbon mobility are preserved.

Conclusions: This study demonstrates an age-dependent decline in ribbon number accompanied by enlargement of surviving puncta, consistent with synaptic remodeling in the aging cochlea. The preserved motility of ribbons across ages suggests that dynamic capacity for movement is maintained, even as synapse density diminishes. These data support a model in which aging preferentially affects ribbon abundance and morphology, rather than mobility. Ongoing work incorporating auditory brainstem response (ABR) measurements will link these synaptic changes to hearing function. Together, these findings highlight presynaptic ribbon remodeling as a potential contributor to age-related auditory decline and establish live imaging of RIBEYE-tagRFP cochleae as a powerful tool for studying mechanisms of synaptic aging.

SU148. Rapid Peri/Post-Mortem Technique for Harvesting and Dissecting Human Temporal Bones That Circumvents Extended Decalcification

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Background: The otic capsule, the densest bone in the body, encases the inner ear structures the cochlea, vestibule, and semicircular canals. Postmortem temporal bone processing typically requires slow fixation and prolonged decalcification taking weeks to months, which can compromise molecular integrity. Here, we present a novel method for rapid fixation and whole-mount dissection of the human cochlea and vestibular end-organs harvested peri/postmortem from human organ donors. This approach minimizes decalcification time, preserves the tissue's structural integrity, facilitates rapid histological analysis, and enables future proteomic and multi-omic studies.

Methods: We have developed an efficient workflow, in collaboration with the federally designated organ recovery organization for Maryland, Virginia, and Washington, DC to harvest temporal bones from organ donors within a very short peri/post-mortem interval of 1 to 2 hours. Using an extracranial approach with a bone plug autopsy saw, specimens were collected and fixed in 10% formalin after disarticulating the stapes footplate. The preparation was subsequently trimmed using a surgical drill in two stages: first, the bone plug was reduced to the otic capsule; second, the various neurosensory tissues were collected, beginning with the vestibular organs (the three cristae, utricle, and saccule) and concluding with delicate drilling around the cochlear capsule, followed by dislocation of the cochlear sensory epithelium from the modiolus. A brief 24-hour decalcification in 14% EDTA was then performed to decalcify the spiral laminae and otoconia.

Results: Whole-mount cochlea with minimal mechanical damage was obtained, as well as round window membrane, ossicles, and spiral ganglion neurons from the Rosenthal canal. In addition, all five vestibular organs—the lateral, anterior, and posterior cristae, the utricle, and the saccule—were harvested. These structures were imaged under light microscopy generating a unique image library of human inner ear structures and nerve fibers. The resulting tissue is suitable for histology, immunostaining, and multi-omics analysis.

Conclusions: This method enables successful harvest of intact cochlear and vestibular organs within hours of death, eliminating the need for extended decalcification, and preserving tissues for histology and multi-omic analyses. This represents a significant advance for human inner ear research.

SU149. Anatomical Integrity of the Human Organ of Corti Visualized Using Paired Optical Coherence Tomography and Histology

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Background: Human cochlear microstructures are delicate and hard to access, making it difficult to noninvasively image intracochlear anatomy and pathology. Towards this need, optical coherence tomography (OCT) has been proposed to visualize OoC structures in situ in fresh human cochleae through the intact round window membrane (RWM). However, granular OCT images cannot reliably identify the distinct cell types that make up the OoC. To more confidently interpret OCT images, we analyzed histology obtained from the same donor for direct comparison between OCT features and histologic structure.

Methods: Pairs of fresh temporal bones (4-24 hours postmortem) were obtained with IRB approval from the Mass General Hospital from donors without a history of unilateral ear disease. One ear was prepared for OCT imaging and the contralateral ear was immediately fixed. After the middle ear cavity was surgically opened to access the cochlea, OCT images were collected from unfixed cochleae through the intact RWM using a 900-nm center wavelength OCT system (Gan6201C1, Thorlabs, Germany). Time of imaging was 10-28 hours postmortem. Following imaging, both ears were histologically processed using a rapid, non-decalcifying histological processing workflow developed in our lab: whole bony specimens were fluorescently labeled and embedded in resin (Technovit 9100NEW®). Embedded blocks were oriented to align the histological sectioning plane with the OCT imaging plane using a customized disc sander. Thin (~100 µm) sections were produced with precision diamond wire saws, followed by confocal and super-resolution microscopy to visualize cochlear microstructures. OCT–histology registration was performed at matched cochlear locations, with 3D reconstructions used to confirm correspondence.

Results: OCT images and histological sections from the same cochlear location across paired human temporal bones were obtained. There was high contralateral symmetry in cochlear morphology within individuals. Direct registration enabled confident interpretation of granular OCT features; e.g., differentiating intact versus collapsed OoC architecture and recognizing

regions indicative of cell loss, all based on histologic ground truth. These comparisons establish OCT signatures for the human OoC that correspond to graded microanatomical pathology.

Conclusions: Paired OCT images and histology registered from the same donor showed similar OoC anatomy and verified our ability to accurately interpret OCT images. OoC anatomy across individuals varied, and pathological states could be differentiated. These results demonstrate the capability of OCT to non-traumatically visualize cochlear integrity through the RWM, highlighting its potential as a diagnostic tool. The ability to determine the presence of hair cells and supporting cells in live patients would enable more precise individualized assessment with potential to guide emerging therapies (e.g., gene- and regenerative approaches). In vivo OCT could assist in targeted therapies, determining anatomic barriers to local delivery such as false round-window membrane, and prevent trauma such as fractures of the osseous spiral lamina.

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SU150. The Inner Ear in NF2-Related Schwannomatosis: Histological and Molecular Perspectives

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Background: NF2-SWN is a rare autosomal dominant genetic disorder characterised by bilateral vestibular schwannomas and progressive hearing loss. It results from pathogenic variants in the NF2 gene, located on chromosome 22q, which lead to loss or dysfunction of the tumour suppressor protein Merlin. Although vestibular schwannomas commonly occur as sporadic, unilateral tumours, NF2-SWN is distinguished by its inherited nature and earlier onset. While tumour compression of neural structures is well recognised, mechanisms of sensory loss beyond tumour burden remain unclear. We are currently studying archival human temporal bones (Brown et al., Hearing Research, 2025) and a mouse model to investigate inner ear pathologies in NF2-SWN.

Methods: Human temporal bone sections stained with haematoxylin and eosin were assessed for cochlear and vestibular degeneration using light microscopy (subject ages 14-65; samples held under UK Human Tissue Authority licence #12161). A conditional knockout mouse model (Postn-Cre; Nf2flox/flox; Gelhausen et al., J Neurosci, 2015) was used to examine inner ear pathology via immunofluorescence. Antibodies targeting endogenous total merlin (DS3SW, Cell Signaling Technology), Aldh1a1 (tumour marker), and Iba1 (macrophage marker) were used to identify molecular and cellular changes. Cre-negative littermates served as controls.

Results: Human samples with bilateral vestibular schwannomas showed widespread degeneration of cochlear and vestibular structures, including loss of spiral ganglion neurons and nerve fibres, disorganisation of the organ of Corti, and Schwann cell proliferation with subsequent nerve compression. Some tumours had a complex composition, with evidence of mixed schwannomas and meningiomas. In 3-month-old Postn-Cre; Nf2flox/flox mice, increased localised expression of Aldh1a1 (but without evidence of tumour growth) and increased numbers of Iba1-labelled macrophages within neural tissues possibly indicated early inflammatory and metabolic stress responses. These changes were absent in Cre-negative controls.

Conclusions: These findings suggest that hearing loss in NF2-SWN may begin with subtle, tumour-independent changes within the cochlea and auditory nerve, linking NF2 gene loss to early sensory dysfunction. Elevated Aldh1a1 expression and increased macrophage indicate that inflammatory and structural mechanisms are active prior to tumour progression. Together, these processes likely contribute to hearing loss and other symptoms. A multi-layered therapeutic approach – targeting both early, non-tumour related changes and later tumour-driven effects – may more effectively preserve hearing and improve outcomes in NF2-SWN.

SU151. Loss of Orexin 2 Receptor Signaling Exacerbates Noise-Induced Hearing Loss

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Background: Orexins, also known as hypocretins, are neuropeptides produced by hypothalamic neurons involved in the sleep-wake cycle, food intake, and autonomic output. We discovered that orexin ligands and receptors are also expressed in the cochlea. Mutation of orexin 2 receptor (Ox2R) leads to early onset narcolepsy. Dual orexin receptor antagonist (DORA) medications are used to treat insomnia with new drugs targeting orexin signaling being tested. Given the previously unrecognized expression of orexin signaling molecules within the cochlea, constitutive loss or knock down of orexin through prolonged DORA medications could alter mechanisms underlying normal hearing sensitivity and/or resilience against damage. We begin to address this by examining hearing in constitutive Ox2R mouse mutants subjected to noise.

Methods: Ox2R wild-type (WT) and knockout (KO) mice were used to investigate the role of Ox2R in moderate noise-induced hearing loss. We used Auditory Brainstem Response (ABR) testing and anatomical assessments to analyze effects of noise on physiological and morphological integrity of the cochleae of Ox2R WT and KO mice. Half of the mice (WT and KO groups) were noise exposed (97dB SPL, 8-16kHz, 2h). ABRs were performed before noise exposure to measure baseline hearing thresholds and post-noise periodically through 21 days to track hearing threshold recovery on all mice. A second cohort of WT and KO mice, under identical conditions as the first, underwent transcardial perfusion (4% paraformaldehyde) 5 days post-noise exposure. The temporal bones of WT and KO mice were isolated, processed, cryostat-sectioned, and hematoxylin and eosin (H and E) stained. Immunohistochemistry was performed on alternate sections using primary antibodies (Ox1R, Aqp1, F4/80) and appropriate secondary antibodies. Photomicroscopy was performed (Leitz DSM and Zeiss LSM880 Confocal).

Results: Hearing thresholds of Ox2R WT noise-exposed mice recovered after 5 days post-noise while threshold shifts in Ox2R KO noise-exposed mice remained elevated and began to decline at 7 days post-noise. Ox2R WT thresholds returned close to baseline while Ox2R KO thresholds did not decline to similar levels by day 21 post noise, indicating that a noise-induced temporary threshold shift in WTs became a permanent shift in KOs. In noise-exposed KO mice, there were morphological changes (H and E) in the stria vascularis, decreased protein localization of i) Ox1R in supporting cells and ii) aquaporin 1 in the spiral ligament, and increased F4/80 localization in the spiral ligament.

Conclusions: The functional and anatomical hearing-related changes observed in our mouse model of constitutive Ox2R loss-of-function may reflect constitutive gene loss in human

narcolepsy and could mirror effects following potential long-term use of DORA medications. The effects on hearing may be predictive of patients who are using drugs long-term which alter the orexin signaling system, potentially putting them at risk for augmented hearing loss.

SU152. The Auditory-Nerve Initial-Peak (ANIP) Response to High-Level Clicks is From a Unidirectional Stereocilia Deflection Away From the Nearest Saturation of OHC Mechano-Electric Transduction

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Background: The auditory-nerve initial peak (ANIP) response is a mysterious response that doesn't fit with most conceptions of how the cochlea works. It is the earliest response from moderate-to-high-level rarefaction clicks in cat auditory-nerve (AN), type-1 fibers with characteristic frequencies (CFs) < 3 kHz. It was first shown to be unusual by its strong inhibition by stimulation of medial-olivocochlear (MOC) efferents, which contrasted with the lack of MOC inhibition of the first peak of basilar membrane click responses. The MOC inhibition of ANIP indicates that the ANIP response is affected, or generated, by outer-hair-cell (OHC) motility. Consistent with this, the ANIP response was shown to be suppressed when high-level, low-frequency "bias" tones quasi-statically pushed the OHC mechano-electric transduction (MET) function into low-slope, saturating edge regions. Contrary to prior theory, our preliminary results showed that the bias-tone phase that produced the most suppression (the major-suppression phase) was opposite for ANIP responses compared to low-level click or tone responses.

Methods: In anesthetized cats, we recorded single-fiber AN responses, in response to low-level clicks, low-level tones, and ANIP-producing, higher-level clicks, with/without 50 Hz bias tones. We used new methods to exclude the AN-fiber excitation from high-level bias-tones, so that this excitation doesn't obscure bias-tone suppression of AN responses.

Results: The major suppression phases for ANIP responses were nearly opposite (~180 degrees different), compared to those of low-level clicks and tones.

Conclusions: An "ANIP-MET-asymmetry hypothesis" is consistent with the results: Low-level responses take several cycles to build up and depend on the local slope in both directions of the MET function; their major suppression phase is when the bias tone quasi-statically deflects the MET operating point to its nearest low-slope, saturation edge. In contrast, while ANIP responses oscillate for many cycles like low-level responses, ANIP is only the first half cycle and requires an OHC stereocilia deflection only in one direction. When a bias tone puts the OHC-MET operating point at its nearest saturating edge, low-level, multi-cycle responses are suppressed, but a rarefaction-click's unidirectional initial stereocilia deflection is away from this saturation, enabling a large traverse of the MET function and a large depolarizing OHC current. The ANIP major suppression arises from bias-tone deflections to the MET-function opposite saturation. Our presentation will point out that the ANIP-MET-asymmetry hypothesis fits well with the new theory that cochlear amplification of low-level responses is due to OHC motility changing the local OoC cross-sectional area at a phase that adds energy to the traveling wave in the scala media-tympani fluid. The ANIP-MET-asymmetry hypothesis also fits with low-level responses

being carried primarily by the traveling wave while ANIP is carried by a cortilymph wave, with both waves accumulating gain as they travel to the CF region.

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SU153. Investigation on Organ of Corti Micro-Mechanics and Inner Hair Cell Stereocilia Stimulation Mechanisms Through 3D Finite Element Models of the Mouse Organ of Corti

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Background: The intricate cytoarchitecture of the organ of Corti (OoC) stimulates inner hair cell (IHC) stereocilia, triggering mechano-electrical transduction (MET) channels. Recent advancements in Optical Coherent Tomography (OCT) technology allow detailed motion measurement of the tectorial membrane (TM) and reticular lamina (RL) relative to the motion of basilar membrane (BM) in both transverse and radial directions. High speed camera imaging also revealed stereocilia motion relative to RL radial direction motion. These experimental data enable modeling effort to capture and explain OoC micro-mechanics, but most modeling effort still lack such details. A slice modeling technique using a 3D Finite Element Model (FEM) for a cochlea with Floquet boundary conditions meets the required details with reasonable computational cost (Wang and Puria, 2025, HR).

Methods: A 3D Finite Element Model (FEM) of a slice of the mouse OoC of the apex was built. The cellular structure of the OoC is reconstructed based on 2-photon measurements on fresh tissue (Soons et al. 2015), as well as Scanning Electron Microscopy and histology slices on fixed tissues (Davies and Forge 1987, Taylor et al. 2012). The model captures the realistic representation of the Deiter cells, Outer Hair Cells (OHCs), IHCs, inner and outer pillar cells, Hensen cells, as well as the fluid space of the inner sulcus, tectorial membrane, and the cortilymph consisting of the inner tunnel of Corti, the space of Nuel, and the outer tunnel. It includes the longitudinal inclination of the outer hair cells, Deiter cells, phalangeal processes and the outer pillar cells resulting in the mosaic pattern of the reticular lamina. The material properties were determined using Machine Learning algorithm to fit experiment data on mouse apex (Lee et al. 2016).

Results: The slice model was able to fit the experimental data of the passive postmortem measurements with reasonable tolerance. The TM and RL motion relative to BM motion were observed. Besides being able to fit the data, we are also able to identify the contribution of the stiffness of each structure, such as TM and RL, on the micro-mechanical motion of the OoC. For example, the stiffness of the OHCs and pillar cells have more significant effect on the motion of the RL than RL stiffness itself. The relationship of the stereocilia motion and its surrounding fluid motion are observed. The two key hypotheses that the model tests are: 1) whether the IHC stereocilia are stimulated by inner sulcus fluid, and 2) the effect of stimulation mode on frequency.

Conclusions: FEM slice cochlear model is a powerful tool to study cochlear OoC micro-mechanics and test IHC stereocilia stimulation mechanism hypotheses. Work supported by R01 DC007910 and R21 DC022922 from the NIDCD of NIH.

SU154. Outer Tunnel Vibration Within the Cochlear Partition of the Basal Turn in Sensitive Gerbil Cochleae

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Background: The outer tunnel is the largest fluid-filled space within the auditory sensory organ, the organ of Corti, in the basal turn of the gerbil cochlea. It consists of motile outer hair cells, the soma and the phalangeal processes of the Deiters' cells. The phalangeal processes connect the somatic cupula of the Deiters' cells to the cuticular plate of the outer hair cells, forming part of the reticular lamina. Despite its structural prominence, the functional role of the outer tunnel in cochlear mechanics remains poorly understood.

Methods: Young Mongolian gerbils of either sex with normal hearing were used in this experiment. The bulla on the left side was opened through a ventrolateral surgical approach. After the stapedial artery was removed from the bony surface of the cochlea, an opening was made in the lateral wall of the scala tympani of the basal turn. When the cochlear partition was positioned approximately in the horizontal plane, the magnitude and phase patterns of the vibrations were measured at the best frequency and frequencies more than one octave below the best frequency from the cross-sections of the outer tunnel and the cochlear partition using a scanning heterodyne low-coherence interferometer.

Results: At the best frequency, all structures of the outer tunnel and the cochlear partition moved coherently in the same direction, with the greatest displacement observed at the top of the outer hair cells. As the sound pressure level increased, the vibration peak extended to other structures, particularly the basilar membrane.

At low frequencies, however, the reticular lamina at the apical end of the outer hair cells and the somatic region of the Deiters' cells moved in approximately opposite directions. Additionally, the top and bottom of the outer tunnel exhibited antiphase motions.

Conclusions: These results suggest that the organ of Corti's response at low frequencies reflects localized, outer hair cell-driven motion, while the response at the best frequency is dominated by amplified traveling waves that mask local cellular activity. When sound-induced length change in outer hair cell alters the outer tunnel's circumference of the outer tunnel, they generate fluid pressure oscillations within the tunnel. Due to the incompressibility of cochlear fluid and the forward-propagating traveling wave, these pressure oscillations in the outer tunnel and other fluid spaces produce longitudinal fluid motion within the cochlear partition. This fluid motion, in turn, enhances the traveling wave, thereby increasing cochlear sensitivity.

SU155. Cochlear Fluid Flow Driven by Active Outer Hair Cells

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Background: Fluids in the inner ear are considered stationary because the inner ear fluid system is nearly isolated from other body fluid systems, such as the cerebrospinal fluid and the blood.

Two lymphatic fluids fill three tube-like cavities in the cochlea. The fourth fluid-filled cavity along the tunnel of Corti is unique to mammals (non-mammalian sensory epithelia do not have such an ample extracellular fluid space). Recent studies have shown that the organ of Corti is 3-dimensionally deformed by active outer hair cells, resulting in a change in the cross-sectional area of its fluid-filled space. We hypothesized that active outer hair cells can induce cochlear fluid circulation.

Methods: Experiment) An excitotoxic drug (10 mM kainic acid) that affects afferent synaptic terminals was delivered to the intact round window of young gerbils. A multi-channel electrode placed at the anteroventral cochlear nucleus measured neural signals while the drug diffused along the cochlea. During the drug delivery, sounds at different levels and frequencies were played. Model simulations) The computational model incorporates structural mechanics, hair cell physiology, and fluid dynamics. Three layers of fluid mechanics were essential to this study: interaction between the scala fluid and the cochlear partition, interaction between the Corti fluid, the organ of Corti and active outer hair cells, and advection-diffusion in the scala and Corti fluids.

Results: Sounds affected cochlear drug delivery. At 50 dB SPL, playing sounds had a minimal effect on the drug's effect time. Between 60 and 80 dB SPL, as the sound level increased, the effect time got shorter. Lower frequency was more effective in facilitating drug delivery. At locations apical to the CF location of sounds, often the delivery took equal or longer time when played loud sounds (< 75 dB SPL). Computer model simulations could explain experimental observations such as the accelerating region (located basal to the CF location of sounds) and the transport barrier (near the CF location).

Conclusions: Our measurements and model simulations show that: 1) Active outer hair cells can drive fluid circulation in the cochlea; 2) Two routes construct the fluid circulation: flux toward the apex along the tunnel of Corti and flux toward the base along the scala tympani; 3) A pure tone stimulus generates an accelerated transport region and a transport barrier.

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SU156. Electrically Evoked Displacements of Outer Hair Cells in the Fresh Cadaveric Human Cochlea

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Background: Prestin, a protein crucial for cochlear hair cell electromotility, has been detected using immunohistochemistry in the lateral cell membrane of outer hair cells (OHCs) in human cadaver cochlear tissue, suggesting its structural preservation even postmortem (Rask-Andersen et al., 2010). Little is known about the human organ of Corti (OoC) displacement generated by the electromotile forces from the OHCs.

Methods: Optical coherence tomography (OCT) was used to image and measure vibrations of the cochlear partition (CP) in the apical region of fresh (< 28 hours postmortem) cadaveric human cochleae in response to acoustic stimulation and electrical stimulation. In all cases, the scala vestibuli was fenestrated for OCT and electrode access. The cochlea was immersed and maintained in chilled artificial perilymph media during preparation and measurements.

Acoustical stimulation was performed with an ear canal probe tube speaker. In one case, the incudostapedial joint was disarticulated and a piezoelectric stack was used to drive the stapes. Electrical stimulation was performed by inserting a glass pipette with a 70 μ m diameter tip into the scala media. The return electrode was placed in the dish. Discrete sinusoidal signals over a range of 100 Hz to 20 kHz were used as a stimulus and the OCT measured displacement response of structures within the CP.

Results: Acoustical stimulation OCT vibrometry results indicated CP tuning of approximately 250 Hz in two preparations that were also subject to electrical stimulation. The electrical stimulation frequency response was more broadly tuned, showing a higher frequency cutoff than the acoustic. With acoustic stimulation, the CP structures moved in phase. With electrical stimulation, out of phase displacement between the basilar membrane and reticular lamina was seen only along the axis of the outer hair cells and Deiters' cells. This suggests that the outer hair cell force effect was activated and localized to the OHCs and Deiters' cells axis. These results are consistent with previously reported measurements in animals (Strimbu et al., 2022, Puria et al., 2025).

Conclusions: Prestin's normal electromotile function requires membrane potential, which is absent postmortem. To overcome this limitation, we introduced a scala media electrical probe, which generated an extracellular potential that partially restores OHC motility in our human cadaver preparation and measured with high resolution OCT.

SU157. Immediate Post-Noise and Short-Time Systemic Elevation of Progesterone Promotes Recovery of Cochlear Synapses From Noise-Induced Synaptopathy in Mice

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Background: Female mice are generally less susceptible than males to noise-induced cochlear synaptopathy (NICS). Remarkably, susceptibility to NICS in females varies through the estrous cycle. Females exposed to noise in the luteal phase (metestrus and diestrus) exhibit reduced synapse loss, while females exposed to noise in the follicular phase (proestrus and estrus) exhibit synapse loss like that in noise-exposed males (Hu et al, ARO-Meeting-2017). Reduced susceptibility to NICS correlates with elevated systemic progesterone (P4) levels, the dominant hormone in the luteal phase, but not with estrogen (E2), the dominant hormone in the follicular phase. To test the hypothesis that P4 could protect against NICS or facilitate recovery from NICSs, P4 was chronically administered via an implanted pellet or acutely administered via subcutaneous injections at various times, pre-noise or post-noise, in CBA/CaJ mice.

Methods: 12-14-week-old males or ovariectomized (OvX) females were exposed to 100 dB SPL, 8-16 kHz octave band noise for 2 hours. Auditory brainstem response (ABR) and DPOAEs were measured at 8, 16, and 32 kHz at three times: pre-noise, post-noise day 1 (PND1), and PND14 to assess baseline thresholds, temporary threshold shifts, and recoveries in a within-subject design. For chronic administration, a P4-pellet (P4P) was implanted subcutaneously, with ABRs measured pre- and post-implantation/pre-noise. For acute administration, water-soluble P4 (P4W) or P4 in sesame oil (P4O) was injected 2-hr pre-noise and/or immediately pre-noise (PrN2, PrN0), and/or immediately or 2-hr post-noise (PN0, PN2). Changes in ABR wave-I

amplitudes at PND14 were quantified as amplitude decline relative to pre-noise. To quantify synapses/inner hair cell, postsynaptic densities, presynaptic ribbons, and hair cells were visualized using anti-PSD95, anti-Ribeye/CTBP2 and anti-myosin antibodies, respectively, in organ of Corti wholemounts at 8, 16, and 32 kHz locations in mice euthanized at PND14.

Results: In P4P OvX females, pre-noise ABR wave-I amplitudes were slightly reduced. Recovery of amplitude on PND14 was not significantly better than nonimplanted controls; synapse counts remained lower than in non-noise exposed controls although slightly higher than nonimplanted controls. In acute P4W or P4O groups, post-noise recovery of ABR amplitudes and synapse numbers were better than the saline control, with no difference between males and OvX females. In both males and OvX females, P4O administered at PN0-only showed similar results to P4O at pre- plus post-noise and better recovery than PrN-only in amplitude recoveries and synapse counts. We did observe increased risk of PTS associated with P4 administration.

Conclusions: P4 ameliorates NICS assessed by ABR amplitude and synapse counts. This appears to be due primarily to facilitating post-noise recovery rather than protection during noise. Chronic P4 delivered from an implanted pellet provides relatively little recovery from NICS relative to acutely-delivered P4 administered by injection following noise exposure. (Supported by NIDCD R01 DC015790)

SU158. Transient Transfection of Fibroblasts to Produce Bdnf on the Surface of Cochlear Implants

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Background: After cochlear implantation, connective tissue is formed around the implanted electrode array. Despite many different efforts to reduce connective tissue formation, some cells will always remain on the electrode surface. Furthermore, the function of a cochlear implant (CI) is relying on the presence of intact spiral ganglion neurons (SGN). Survival of SGN can be supported by the application of neurotrophic factors such as brain-derived neurotrophic factor (BDNF). The aim of our approach is to modify existing fibroblasts on the surface of a CI electrode array to produce and deliver BDNF. This production on the electrode surface could also provide a directional cue in case new neurites are formed by SGN.

Methods: Two recombinant vectors coding for td-Tomato and BDNF (5.6 and 8.8 kbp) were used to transfect NIH/3T3 cells. The plasmids were complexed using different amounts of branched 25 kDa polyethyleneimine (PEI). Complex formation (size and surface charge) was investigated using a zetasizer for different ratios of PEI and DNA. The substances alone as well as the complexes were tested regarding cytocompatibility using cell viability tests. For transfection, the cells were incubated with the complexes for 3 hours before further cultivation. Transfection efficiency was determined 24 hours after starting the incubation by comparing cells exhibiting red fluorescence to the total number of cells.

Results: Complexes between DNA and PEI were successfully formed. Up to an amount of PEI of 1000 ng per well, the complexes appeared to be cytocompatible. Transfection efficacy at 24 hours after start of the incubation was below 1% for the large vector and close to 10% with the

smaller vector with the highest values for equal amounts of DNA and PEI in the solution. The complexes could be incorporated into polymeric coating that can be used for cochlear implant electrode arrays.

Conclusions: With the smaller vector, a system to transfect fibroblasts to produce and deliver BDNF is available that can potentially be combined with CI electrodes. This system can now be used to investigate further questions such as release from the coating, duration of transfection etc.

SU159. Clustering Analysis Reveals Distinct Macrophage Phenotypes Following Excitotoxic Injury in the Zebrafish Lateral Line

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Background: Macrophages exhibit diverse morphological and motile features that reflect distinct responses to cochlear injury and subsequent synaptic repair. Recent studies in mice demonstrate that macrophages are recruited to the cochlea after moderate-intensity noise exposure, but the dynamic transitions between macrophage states during excitotoxic injury remain poorly understood. Real-time visualization of macrophage-synapse interactions in the intact mammalian inner ear is challenging, but the zebrafish lateral line provides a powerful model to enable fluorescent labeling and time-lapse imaging of macrophage behavior in vivo. Here, we employed live imaging and unsupervised clustering of macrophage tracks and features to identify and characterize discrete macrophage phenotypes following excitotoxic injury.

Methods: 6-7 day old transgenic zebrafish expressing a red fluorophore in macrophages (tg(mpeg1:tdtom)) and either GFP in afferent neurons (tg(neurod:GFP)) or a cytosolic targeted Ca²⁺ indicator (tg(hsp70:GCaMP6s:sill1)) were imaged with a spinning disc confocal microscope. Fish were paralyzed with α -bungarotoxin and mounted in an imaging chamber before visualizing the neuromast hair cells and afferent neurons. After obtaining baseline images, fish were exposed to 100-300 μ M AMPA for 15-30 minutes to simulate excitotoxic injury and were then rinsed with embryo media. Time-lapse imaging followed for 2-3 hours, and results were compared to control fish without AMPA exposure. Automated tracking (FIJI-Trackmate) and unsupervised clustering analysis (CellRomeR) were utilized to compare morphologic (area, circularity) and motility (speed, directional change rate) features to identify phenotypic clusters unique to each condition.

Results: Cluster analysis revealed distinct macrophage phenotypes with different prevalence in the control and AMPA-exposed conditions. The control condition was composed primarily of macrophages that were larger and slower without significant directional changes, which was consistent with a “surveillance” phenotype. In the AMPA-exposed group, there were multiple distinct phenotypes with a portion of macrophages adopting the “patrolling” phenotype but a larger proportion that were smaller and faster with a high directional change. Such behavior is consistent with an “activated” phenotype. These experiments are ongoing.

Conclusions: Our findings demonstrate that excitotoxic injury results in shifts between distinct macrophage phenotypes in the zebrafish lateral line. The results highlight the utility of clustering analysis in identifying discrete macrophage subtypes in vivo and for linking dynamic morphologic and motility features to injury. Further work will involve implementing a similar

cluster analysis paradigm for the lateral line imaged with light sheet microscopy after excitotoxic injury.

SU160. Investigating the Role of Neogenin in Cochlear Synaptopathy

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Background: Synapses between cochlear hair cells and Type 1 auditory nerve fibers are the most vulnerable to acoustic trauma, leading to permanent synaptopathy despite recovery of temporary threshold shifts. Previous therapeutic interventions in mouse models have demonstrated a limited window for synaptic recovery. Treatments, such as neurotrophins, administered before or immediately after noise trauma have shown a protective rather than a regenerative effect. However, an antibody targeting Repulsive Guidance Molecule A (RGMa) restored synapses for up to 1 week after noise exposure, suggesting a regenerative effect on the synapse. RGMa is involved in axonal guidance through interactions with neogenin1 (Neo1), a cell surface receptor expressed on auditory nerve fibers. Epigenetic barriers can restrict synaptogenesis in the adult sensory nervous system, limiting the restoration of the cochlear afferent synapse in the noise-exposed inner ear. Here, we hypothesize that RGMa-Neo1 interactions inhibits synaptic repair by facilitating the nuclear translocation of the Neo1 intracellular domain (Neo-ICD), which mediates epigenomic changes after acoustic trauma.

Methods: We developed clonal Neo-HA Neuro-2a cell lines and generated Neo1 and Neo-ICD EGFP tagged constructs to evaluate regulated intramembrane proteolysis and subcellular localization of fragments using immunoblotting and immunofluorescence under different pharmacological conditions. Nuclear activity and chromatin binding of Neo-ICD was assessed in-vitro using Cut and Run assays. To evaluate functional recovery of synapses, we inhibited Neo-ICD cleavage in an ex-vivo kainic acid (KA) excitotoxicity model of cochlear synaptopathy and quantified synaptic counts.

Results: Neo1 undergoes endogenous stepwise proteolysis to release the Neo-ICD in-vitro. We observed that intramembrane proteolysis is mediated by γ -secretase and Neo-ICD undergoes rapid degradation by the proteasome. Nuclear translocation is tightly regulated where nuclear export, but not nuclear import, determines steady state localization. Cut and Run assays revealed that Neo-ICD binds to chromatin with co-localization to histone markers. Preventing intramembrane cleavage of Neo-ICD in ex-vivo KA models showed a dose-dependent effect to regenerate cochlear synapse counts.

Conclusions: Our findings identify neogenin1 as a potential epigenetic regulator of chromatin accessibility and may provide a novel therapeutic strategy to extend the therapeutic window for synaptogenesis in the cochlea.

SU161. Using Simulations and Non-Stationary Fluctuation Analysis of Synaptic Currents to Uncover the Properties of Cochlear AMPA-Type Glutamate Receptors: Ca²⁺ Permeable and Impermeable

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Background: AMPA receptor/channels are concentrated in specific regions of synapses called postsynaptic densities (PSDs) where they mediate fast neurotransmission. PSDs vary in size among synapse type, differing in number, spatial density, and composition of subunits. For spiral ganglion neurons (SGNs) contacting inner hair cells, PSD composition heterogeneity may in part determine sound response properties. However, the number of AMPARs within single PSDs, their spatial organization, and their molecular heterogeneity are currently unknown.

Nonstationary fluctuation analysis (NSFA) has been used to quantify the number of receptors (N) and their unitary currents (i) in synapses of the CNS. By recording and analyzing excitatory postsynaptic currents (EPSCs), NSFA can characterize AMPAR properties. Yet while NSFA outputs a single estimate for i, unitary currents depend on AMPAR subunit composition. Depending on the absence or presence of the GluA2 subunit, AMPARs are calcium permeable (CP-AMPARs) or calcium impermeable (CI-AMPARs), respectively.

To extract distinct i estimates for CP-AMPARs and CI-AMPARs and their relative abundance, here we report on a method of analysis taking advantage of IEM-1925, a selective CP-AMPAR blocker. We will also share the parameters of a simulation matching the kinetics of physiological EPSCs. Finally, we will compare NSFA results on simulated EPSCs (sEPSCs) to NSFA on vesicular EPSCs (vEPSCs), EPSCs evoked by glutamate uncaging (uEPSCs), and vEPSCs of cells treated with IEM-1925.

Methods: Explants of the organ of Corti from P15-P17 C57BL/6 mice were used for patch-clamp recordings. uEPSC recordings were generated via rapid homogeneous uncaging of glutamate on the entire PSD. vEPSCs were recorded before and after treatment with IEM-1925. EPSC simulations and NSFA tools were scripted in Python.

Results: To reproduce the ranges and distributions of amplitudes and kinetics observed in vEPSCs, the simulations required that each EPSC be generated from variable N and [glutamate], defined by normal distributions, fit to an ensemble of physiological data: $\mu=2200$, range=50–3422; and $\mu=2.5\text{mM}$, range=1–5.8mM, respectively.

It is unclear how robustly NSFA can extract N and i in recordings from synapses with such heterogeneous EPSCs. For sEPSCs, some permutations of NSFA produced accurate estimates of i. However, information on N and Popen remain obscure.

For sEPSCs and uEPSCs, where each channel sees glutamate simultaneously, the pattern of EPSC amplitudes and kinetics are similar, but both differ from patterns observed for vEPSCs. Inclusion of a simple model of glutamate diffusion may in part explain these differences.

NSFA revealed significantly lower N and i for IEM-1925 versus control. By comparing mean i between EPSCs recorded under control vs. a saturating concentration of IEM-1925, we extracted i for CP-AMPARs and CI-AMPARs and their relative proportions.

Conclusions: Simulations of EPSCs recorded from SGNs can validate NSFA on physiological data to extract essential channel properties in support of pre-clinical translational research.

SU162. Single-Cell and Spatial Transcriptomic Analysis of Noise-Induced Cochlear Synaptopathy

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Background: Noise-induced hearing loss (NIHL) may arise in part from cochlear synaptopathy, the loss of ribbon synapses between inner hair cells and spiral ganglion neurons (SGNs). This process, thought to be driven by glutamate excitotoxicity, occurs even in the absence of hair cell loss and disrupts the fidelity of auditory signaling. Synaptopathy has been studied as a cause of auditory dysfunction, and has demonstrated tonotopic differences in synapse loss after noise exposure. However, the cellular heterogeneity of SGN responses and the molecular mechanisms that mediate synaptic degeneration and repair remain incompletely defined.

Recent evidence has identified the molecular subtypes of SGNs, which correlate with their electrophysiological properties and susceptibility to excitotoxic injury (Shrestha et al. 2018). There are three Type 1 SGN subtypes (1A-C), with Type 1C being the most susceptible to noise-induced synaptic loss. We aim to determine the underlying molecular responses in noise-induced hearing loss and cochlear synaptopathy using single-cell and complementary RNA in situ hybridization for spatial relationships. We hypothesize that there are SGN-specific and tonotopic-regional differences in synapse related genes after noise exposure.

Methods: A two-tiered approach was used to investigate transcriptional changes after glutamate excitotoxicity and noise-induced cochlear synaptopathy. First, an in vitro 3D cochlear explant model of neonatal mice (P2-3) were exposed to a glutamate agonist or glutamate agonist and neurotrophins to induce synapse regeneration. Following treatment, explants underwent single cell RNA sequencing and computational analysis.

To validate transcriptomic findings in vivo, an established mouse model for noise induced synaptopathy was used. The synaptopathy phenotype was confirmed using ABR/DPOAE measurements and synapse quantification on immunohistochemistry using anti-CtBP2 and anti-GluR2 antibodies. Cochleae were harvested 24 hours post noise-exposure and tonotopically microdissected. Spatial expression of candidate genes was examined using RNA in situ hybridization (RNA-ISH) in a whole mount preparation in the 11.33 kHz and 32 kHz regions. Machine learning-assisted three-dimensional imaging analysis was used for quantification.

Results: Single-cell RNA sequencing of cochlear explants following glutamate excitotoxic injury and neurotrophin exposure yielded high-quality cells across conditions. Clustering identified populations of hair cells and spiral ganglion neurons identified using their canonical markers. After glutamate agonist exposure, there was an increase in the expression of glutamate receptor subunit mRNA and an even greater increase after NT3 exposure. In vivo validation using RNA-ISH in noise-exposed mice confirmed spatially distinct increases in glutamate receptor subunit mRNA by SGN subtype and by tonotopic region.

Conclusions: These data suggest that subtypes of spiral ganglion neurons susceptible to synaptic loss due to noise trauma, respond in turn by generating more synapses. These findings provide a greater insight into the mechanisms that underlie synaptic loss and recovery which could garner targeted treatments for noise-induced hearing loss.

SU163. Glu2Py as a Novel Neurotrophic Agent for Inducing Neuritogenesis in Spiral Ganglion Cultures

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Background: Cochlear synaptopathy, involving the loss of afferent ribbon synapses between cochlear hair cells and spiral ganglion neurons (SGNs), is a leading cause of sensorineural hearing loss and continues to present a major clinical challenge due to the lack of effective therapeutic options. The development of neurotrophic compounds that can promote spiral ganglion (SG) neurite outgrowth and enhance neuronal survival is critical for advancing hearing restoration strategies. Glucosyl-2-pyrrolopyrimidinone (Glu2Py), a novel pharmacologic inhibitor of Misshapen Kinases, has shown potential neurotrophic activity in preliminary studies. However, its specific effects on auditory neurons and nerve fibers remain to be systematically characterized.

Methods: We evaluated the neurotrophic effects of Glu2Py (1, 5, and 10 μ M) using multiple experimental models, including SH-SY5Y human neuroblastoma cells, cochlear spiral ganglion explants, and dissociated SGN cells from postnatal day 3.5 CD1 mouse pups. SH-SY5Y cells were treated with Glu2Py for either 24 or 48 h followed by 48-h recovery periods, while SG tissues were exposed to Glu2Py for 24-48 h depending on the experimental design. Cell morphology measurements and neurite length analysis were performed using fluorescence microscopy and ImageJ software with NeuronJ plugin. Statistical analysis employed appropriate parametric and non-parametric tests based on data distribution.

Results: Glu2Py demonstrated significant dose-dependent and time-dependent neurotrophic effects across all models. In SH-SY5Y cells, 24-h treatment showed concentration thresholds with increased neurite-to-cell outgrowth at 1 (1.00 ± 0.42), 5 (1.23 ± 0.10), and 10 μ M (1.52 ± 0.05) versus untreated cells (0.56 ± 0.14). Neurite outgrowth directly affected mean cell area, confirming treatment effects. Treatments at 1, 5, and 10 μ M significantly increased cell areas (547.95 ± 246 , 599.51 ± 326.21 , and 572.61 ± 296.61 μ m², respectively, $p < 0.0001$) compared to controls (341.49 ± 170.16 μ m²). In 48-h treatment groups, mean area increased at 5 μ M (601.43 ± 308.64 μ m², $p < 0.01$) and 10 μ M (1183.48 ± 442.81 μ m², $p < 0.0001$) versus controls (423.67 ± 111.31 μ m²), while 1 μ M showed no significant effect. Spiral ganglion explants demonstrated robust concentration-dependent neurite outgrowth, with highly significant increases at 5 μ M (94.21 ± 31.39 μ m) and 10 μ M (102.63 ± 33.04 μ m) compared to controls (65.44 ± 14.55 μ m, $p < 0.0001$), while 1 μ M showed no significant effect (59.51 ± 21.91 μ m). Remarkably, separated spiral ganglion cells exhibited enhanced sensitivity, showing significant neurite extension at all concentrations: 1 μ M (81.19 ± 17.62 μ m), 5 μ M (77.55 ± 33.62 μ m), and 10 μ M (99.90 ± 20.01 μ m) compared to controls (53.54 ± 17.92 μ m, $p < 0.0001$). Differential

responses between explant and separated cultures suggest tissue architecture and cellular microenvironment significantly influence compound efficacy and cellular responsiveness.

Conclusions: Glu2Py exhibits potent neurotrophic activity with particular efficacy in promoting SGN outgrowth. The compound shows time-dependent effects in a human neuronal model and potency in primary tissue explants and dissociated SGNs. These findings establish Glu2Py as a promising therapeutic candidate for cochlear neuropathy and synaptopathy, warranting further investigation in ototoxicity protection and regenerative sensorineural hearing loss treatment applications.

SU164. Daily Dynamics of the Electrode-Tissue Interface in Cochlear Implants With and Without Steroid-Eluting Electrodes

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Background: Cochlear implant electrode impedance provides insight into inner ear tissue responses. After implantation, impedance typically rises due to protein adsorption and inflammatory tissue changes, then several changes occur after the onset of electrical stimulation. Excessive impedance growth is linked to fibrotic encapsulation of the electrode and can negatively impact device performance. Steroid interventions (e.g. topical or eluting dexamethasone) have been explored to mitigate these foreign body responses and impedance increases. However, the day-to-day impedance dynamics in the immediate remain poorly characterized. This study aimed to monitor daily impedance changes and their resistive vs. polarization subcomponents in cochlear implant recipients with and without sustained steroid delivery, and to relate these changes to device usage patterns.

Methods: A prospective observational design was used to analyze daily impedance telemetry (morning and night) in cochlear implant recipients. Four groups were studied based on implant type and steroid administration: (1) CI612 electrodes with no dexamethasone application, (2) CI612 electrodes with a single intraoperative topical dexamethasone application, (3) CI632 electrodes with the same topical dexamethasone treatment, and (4) CI632 electrodes incorporating a self-eluting dexamethasone polymer for sustained release. Impedance was measured daily from the day of surgery through the post-activation period using standard clinical telemetry. To dissect impedance components, we calculated the resistive and polarization subcomponents. Patients' device data-logging records (hours of use per day) were also collected in a subgroup to examine the influence of daily cochlear implant use on impedance fluctuations.

Results: Daily impedance monitoring revealed consistent temporal patterns across patients. As expected, impedance increased during the early postoperative period and stabilized over time. Significant differences were observed across electrode types and dexamethasone delivery methods. After activation, clear diurnal fluctuations emerged, primarily driven by changes in the polarization component, while the resistive component remained stable. The self-eluting dexamethasone electrode exhibited the most stable profile, significantly reducing day-to-night changes and flattening the polarization impedance curve. Differences in the resistive subcomponent were minimal across groups. Notably, patients with greater daily CI use showed larger diurnal impedance shifts, suggesting that electrical stimulation modulates the electrode-tissue interface.

Conclusions: Our findings demonstrate that the polarization component of electrode impedance is highly sensitive to changes in cochlear implant (CI) stimulation and is significantly modulated by anti-inflammatory interventions. Dexamethasone, particularly when delivered via a self-eluting electrode array, not only attenuates the post-surgical rise in impedance but also reduces day-to-day variability, leading to a more stable electrode–tissue interface. In contrast, the resistive component remains largely unaffected by both steroid intervention and electrode type. From a clinical perspective, daily impedance monitoring offers a high-resolution window into cochlear healing and tissue response. When combined with targeted steroid delivery, this strategy may enable earlier detection of abnormal biological responses and support more personalized postoperative management.

SU165. Feasibility of Endoscopic Microneedle-Guided Round Window Membrane Perforation and Perilymph Sampling in Human Cadaveric Temporal Bones

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Background: Diagnostic and therapeutic interventions for inner ear disorders remain limited by the lack of safe, controlled access to the perilymphatic space. Haystack Medical has developed a microneedle-endoscope system designed for minimally invasive round window membrane (RWM) perforation and fluid aspiration under direct visualization.

Methods: Hollow microneedles (outer diameter 100 μm , lumen diameter 35 μm) were fabricated by two-photon lithography and mounted onto polyimide tubing. A refined deployment tube (23 Ga) enabled endoscopic visualization and precise positioning adjacent to the RWM. Cadaveric human temporal bones (n=7) were used to evaluate procedural feasibility. Following endoscopic RWM perforation, perilymph aspiration was performed using a syringe pump at $\sim 1 \mu\text{L}/\text{min}$. Aspirated volumes were quantified microscopically via droplet measurement.

Results: Controlled, reproducible RWM perforation was achieved in all specimens (100% success, n=7) without tearing or enlargement of the membrane. Aspiration was successful in all cases, with mean volume 0.89 μL (SD 0.20 μL), slightly below DI water controls (1.0 μL). Needle integrity was preserved after each use, and no uncontrolled RWM damage was observed. Some aspirates contained visible blood contamination, likely attributable to cadaveric conditions.

Conclusions: Haystack Microneedles enabled consistent, visually guided perforation and reliable perilymph aspiration in human cadaveric models. Achieved aspiration volumes are sufficient for biomarker analyses, demonstrating feasibility of minimally invasive diagnostic sampling. These Phase I outcomes establish a strong foundation for clinical translation and regulatory preparation.

SU166. Development of a Drug-Induced Ototoxicity Model Using Inner Ear Organoids and Investigation of Protective Mechanisms

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Background: Ototoxic drugs such as aminoglycoside antibiotics and cisplatin are widely used in clinical practice

but are a major cause of irreversible hearing loss, primarily through damage to cochlear hair cells.

Current ototoxicity studies primarily rely on animal models or two-dimensional cultures, which fail

to adequately reproduce the structural and functional complexity of the human inner ear. Human pluripotent stem cell–derived inner ear organoids represent a physiologically relevant platform with the potential to model both auditory development and drug-induced damage. The aim of this study is to establish an inner ear organoid–based ototoxicity model and to explore protective mechanisms through the modulation of key signaling pathways, including Wnt, PI3K/PTEN, and

YAP

Methods: Human embryonic stem cells and induced pluripotent stem cells were differentiated into inner ear

organoids using protocols established in our laboratory. This process involved the induction of otic placode formation, vesicle development, and sensory epithelium differentiation. Organoids are

characterized by immunostaining and molecular assays for lineage-specific markers, including ATOH1, POU4F3, and MYO7A (hair cells), as well as SOX2 and PROX1 (supporting cells). To model

ototoxic injury, organoids will be treated with aminoglycoside antibiotics and cisplatin, followed by

evaluation of cell survival, ROS generation, and morphological changes using immunostaining and

LDH assays. Protective strategies will be tested by applying small molecules, including the Wnt activator CHIR99021, the PTEN inhibitor VO-OHpic, and the ROS scavenger N-acetylcysteine.

Transcriptomic profiling via RNA-seq will be performed to identify molecular pathways involved in

damage and recovery.

Results: Preliminary experiments have focused on directing human pluripotent stem cells toward inner ear

organoid differentiation. Organoid formation efficiency is currently being systematically evaluated

across multiple cell lines, and immunostaining is being used to quantify the proportion of organoids expressing otic lineage markers. Ototoxic drug treatment has not yet been initiated; protocols for exposure to aminoglycoside antibiotics and cisplatin are under development. Comprehensive quantitative analyses will be conducted in the next phase, and results will be presented at the meeting.

Conclusions: This study highlights the potential of human pluripotent stem cell–derived inner ear organoids as

a physiologically relevant model for investigating drug-induced ototoxicity. Modulation of Wnt, PI3K/PTEN, and YAP signaling pathways is expected to provide novel insights into protective mechanisms for auditory hair cells. Such an approach may contribute to the establishment of a precise preclinical drug-screening platform and facilitate the development of innovative therapeutic strategies aimed at preventing or reducing hearing loss induced by ototoxic agents.

SU167. A Novel Device for Atraumatic Perilymph Sampling

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Background: A promising approach to investigating cochlear physiology is the analysis of perilymph, a sodium-rich extracellular fluid present in the cochlea and vestibular system with interaction with key auditory structures. Because of its anatomically challenging location and the immediate risk of irreversible hearing loss following cochlear injury, perilymph sampling in humans is currently only feasible during surgical procedures that are already expected to result in hearing loss, such as cochlear implantation or removal of vestibular schwannoma. In contrast, animal models allow the evaluation of innovative approaches for perilymph collection. Several techniques are available for obtaining perilymph from animal models, varying in their degree of invasiveness and potential to cause irreversible damage. The choice of methodology should be guided by the specific scientific objective, with the aim of collecting perilymph as atraumatically and uncontaminated as possible to ensure its validity as a diagnostic tool.

Methods: In three pigs (*Sus scrofa domestica*), surgical access to the inner ear was established, and the round window membrane was exposed. A novel sampling device was advanced through the round window membrane to collect perilymph. In two animals, auditory brainstem responses (ABRs) were recorded both preoperatively and one week postoperatively to assess potential changes in auditory function resulting from the procedure. Following functional assessment, the inner ears were harvested and subjected to high-resolution micro-computed tomography (micro-CT) analysis. To confirm that the collected fluid was indeed perilymph, the presence of cochlin was verified using an enzyme-linked immunosorbent assay (ELISA).

Results: After surgical exposure of the round window, perilymph was successfully collected using the novel device. In three samples, a volume of 3–5 µL was obtained, in which the presence of cochlin was clearly confirmed via ELISA. The structural integrity of the cochlea

remained largely preserved, as shown in the micro-CT data. In the two animals monitored for one week postoperatively, minor shifts in hearing thresholds were observed in some instances, with an average change of 5 dB across all frequencies.

Conclusions: Atraumatic collection of perilymph using the novel device was successfully demonstrated in a large animal model. The procedure required minimal training for surgeons and allowed the collection of sufficient volumes for diagnostic and analytical purposes. Although slight shifts in hearing thresholds were observed, these were minimal and cannot be conclusively attributed to the device itself, as the surgical procedure inherently carries a risk of minor threshold changes. Micro-CT imaging revealed only limited structural alterations at the round window membrane, suggesting favorable conditions for subsequent healing. To further assess recovery and long-term outcomes, studies with extended follow-up periods are warranted.

SU168. Perilymph-Sampling: A Translational Journey

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Background: Translational cochlear implantation, gene therapy and pharmacological distribution strategies require robust animal models to effectively mimic human cochlear anatomy and physiological responses, as well as a similar genetic profile. The pig has emerged as an ideal large animal model due to its anatomical and physiological similarities to the human inner ear. Here, we summarize recent advancements and outcomes from out pig-based cochlear implantation, gene and drug delivery studies providing crucial translational insights.

Methods: We established and continually refined a cochlear implantation surgical protocol in pigs designed to replicate human cochlear anatomical dimensions. This approach facilitates the examination of trauma induced by electrode insertion and optimizes implantation techniques for clinical translation. Drug delivery studies, conducted using various infusion methods with and without CE-certified catheters, provided critical insights into fluid dynamics within the large cochlea.

Results: Utilizing robotic-assisted cochlear implantation, we observed significant reductions in electrode impedance and improved hearing preservation compared to conventional manual insertions, highlighting the potential of robotic assistance to enhance implantation outcomes and reduces intracochlear trauma. We developed and implemented comprehensive perilymph sampling protocols in the pig, including traumatic and atraumatic round window membrane sampling, stapedotomy and stapes fenestration, semicircular canal opening and apical sampling. These methods enabled detailed proteomic and transcriptomic characterization of perilymph before and after cochlear implantation. Notably, our data represents the first description of porcine cochlear expression profiles, providing unique opportunities for comparative analysis with human perilymph expression data. Additionally, we assessed cochlear distribution and off-target effects of various viral vectors, particularly adeno-associated viruses (AAV). Our results from gene delivery studies demonstrated successful cochlear transduction without requiring fenestration, suggesting that intracochlear administration of AAVs is feasible and effective

across the entire cochlear length. However, distribution patterns varied considerably between the chosen vector and the methodology of injection.

Conclusions: Data generated from porcine studies offer a high degree of translationality to clinical application. Differences in transduction from gene delivery studies correlate indirectly with functional outcomes in ongoing otoferlin gene therapy studies, emphasizing the importance of vector selection and delivery precision.

Collectively, these findings underscore the pig's model utility in translational auditory research, providing pivotal insights that advance multiple fields from cochlear implantation to gene therapy strategies for hearing loss treatment.

SU169. Histamine Receptors in the Auditory Midbrain and Thalamus of the Mouse

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Background: The central histaminergic nervous system, originating from the tuberomammillary nucleus (TMN) of the hypothalamus, widely innervates almost the whole brain including the midbrain and thalamus. Brain histamine regulates diverse physiological functions, including wake and arousal states, forming part of the ascending reticular activating system. While the modulatory role of histamine in the brain, particularly in the cerebral cortex, is widely explored, very little is known about its function in the auditory system; in particular, histamine's role in the auditory midbrain (inferior colliculus: IC) and auditory thalamus (medial geniculate body: MGB) has been largely unexplored

Methods: Therefore, to assess histaminergic innervation of these auditory structures, we neuroanatomically and physiologically assessed IC and MGB neurons for functional histamine receptors. We immunohistochemically stained for histamine receptor subtypes (H1R, H2R, H3R) in both the IC and MGB. In addition, we employed in vitro whole-cell patch-clamp to record from IC and MGB neurons in response to bath application of histaminergic agonists.

Results: From our studies, we find a unique distribution of histamine receptors in the IC and MGB of the mouse that correlates with the responsiveness of neurons to histaminergic agonists, which in general act to depolarize auditory neurons

Conclusions: Overall, our data suggests that histamine can selectively heighten the responsiveness of auditory thalamic and midbrain structures to acoustic signals and highlights the overlooked role of histamine in these structures of central auditory processing

SU170. Acetylcholine Release in the Inferior Colliculus During a Stimulus-Specific Adaptation Paradigm

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Background: Stimulus-specific adaptation (SSA) is a neural process that supports novelty detection by selectively diminishing responses to repeated sensory stimuli. Detection of novel

sounds is important for survival and communication, but the neural mechanisms underlying novelty detection in the central auditory system remain largely unclear. The inferior colliculus (IC) serves as a major hub for auditory information integration and is the first auditory subregion to exhibit SSA in the ascending auditory pathway. Additionally, exogenous application of acetylcholine (ACh) reduces SSA in the IC by enhancing responses to repeated sound stimuli (Ayala and Malmierca, 2015). Previous anatomical studies indicate the IC receives dense cholinergic innervation from the pedunculopontine tegmental nucleus (PPN), a key component of the reticular activating system that influences arousal, attention, and sensory processing. Several studies have indicated that PPN neurons respond to sounds and rapidly adapt to repeated sounds, but how cholinergic signaling from the PPN shapes responses in the auditory system remains largely unknown. We hypothesize that cholinergic signaling from PPN projections to the IC modulates SSA in the IC. As the first step in testing this hypothesis, here we examine how novel and repeated sounds shape ACh release in the IC.

Methods: We are currently investigating ACh release in the IC using in vivo fiber photometry in mice. Adult P60-P90 B6.CAST-Cdh23Ahl⁺ mice, balanced for sex, are injected in the IC with an adeno-associated virus encoding the fluorescent ACh sensor, GRAB-ACh3.0 (GPCR-Activation-Based-ACh), and implanted with an optical fiber in the IC. Several weeks later, mice are head-fixed in a sound-isolation booth and exposed to a pure-tone oddball stimulation paradigm, consisting of standard tones at 70 dB SPL for 200 ms for 90% of trials pseudorandomly intermixed with deviant tones at 70 dB SPL for 10% of trials. Interstimulus intervals vary by 2-5s for a 30-minute recording period. Fluorescent GRAB-ACh3.0 signals are analyzed to determine the amount of ACh release evoked by the standard and deviant tones.

Results: Thus far, we have successfully expressed AAV9-hSyn-GRAB_ACh3.0 in the IC and confirmed that GRAB-ACh3.0 fluorescent signals change in response to novel acoustic stimuli. Experiments to assess how ACh release in the IC changes during SSA stimulus paradigms are currently underway.

Conclusions: This project aims to elucidate how acoustic stimuli influence the release of ACh in the IC and the role of cholinergic signaling in the IC in SSA. We expect our results will show that novel sound stimuli elicit more ACh release in the IC than repeated sound stimuli. We will next use optogenetic tools to manipulate cholinergic PPN projections to the IC to test how inhibiting and enhancing these projections alters IC neuron responses to novel and repeated stimuli.

SU171. Decoding Single and Multi-Neuron Activity in Inferior Colliculus for Speech Digits in Natural Environmental Noise

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Background: Inferior colliculus (IC) is one of the brain's crucial auditory processing and integration centers. Previous results suggest that IC may play a key role in the representation of spectral and temporal modulation statistics (Zhai et al., 2020), and computational models of IC have recently been shown to predict how different background sounds impact speech recognition accuracy (Clonan et al. 2025). Although previous work in IC has examined on how simple sounds, such as tones, are segregated from background noise, how IC contributes to the encoding

and segregation of speech in the presence of natural background sounds is not yet well understood.

Methods: This project aims to characterize how spectrum and modulation cues in natural background sounds influence the representation of speech in IC activity of un-anesthetized rabbits. Using an stimulus paradigm based on a previous study in human listeners (Clonan et al 2025), we delivered speech digits (0 to 9) embedded in 11 original (OR) natural background sounds and perturbed variants where we either preserved the spectrum (spectrum matched, SM) or modulation (modulation matched, MM) statistics of each sound at 0 dB signal-to-noise ratios (SNR). We then use a decoding approach to classify digits based on single neuron recordings from IC was obtained while animals listen to these speech-in-noise stimuli. Using Spike Distance Metric classifiers that analyze spike trains from individual or multiple single neurons at different time-scales we evaluated how IC responses contribute to speech identification.

Results: As expected, classification accuracy improved with increasing number of neurons. While the single- and multi-neuron digit classification accuracy for clean speech was often very high (~99%), accuracy for speech-in-noise varied widely depending on the background. Accuracy varied substantially from background to background and was generally highest for SM backgrounds and lowest for MM. We observe that the discriminability of spike trains peaks at frequency bands with center frequency of ~4 Hz and ~100 Hz, corresponding to word rates and periodicity pitch frequencies of the speech vocalizations.

Conclusions: The general classification accuracy trends for OR, SM and MM backgrounds are correlated with those observed in human behavior (Clonan et al 2025) suggesting that spectrum and modulation statistics of each background influence decoding accuracy. Furthermore, the results indicate that rhythm (~4 Hz) and periodicity pitch (~100 Hz) bands may play a critical role in the subcortical representation of speech. Funded by NIH (R01DC020097).

SU172. Characterization of Subcortical Presynaptic Inputs to Inferior Colliculus Subdivisions

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Background: The inferior colliculus (IC) is a mandatory relay station in ascending auditory pathways and plays an important role in sensory integration. Classically, the IC is divided into core (lemniscal) and shell (non-lemniscal) regions, which differ in sound response properties and connectivity. While projections to the core, or central nucleus (CIC), are well established across species, inputs to the shell, or dorsal (DCIC) and external (ECIC) cortices, remain understudied. Furthermore, our recent work showed that rostral and caudal ECIC subregions relay ascending inputs to distinct thalamic targets with different latencies, raising the possibility that they may convey specialized information from distinct sources.

Methods: In this study, we quantified ascending inputs to the IC subdivisions by performing monosynaptic retrograde tracing from CIC, DCIC, rostral ECIC, and caudal ECIC using fluorophore-conjugated wheat germ agglutinin in mice. Coronal brain sections were aligned to a brain atlas for systematic quantification of input cell distributions.

Results: Preliminary results show distinct subcortical input patterns across subregions. Inputs to CIC were largely consistent with previous literature, with dense projections from the ventral cochlear nucleus, intermediate and ventral nuclei of the lateral lemniscus, and the lateral superior olive. In contrast, shell regions received broader inputs, including strong projections from the paralemniscal nucleus, dorsal and intermediate nuclei of the lateral lemniscus, and additional contributions from motor-related areas in the medulla, pons, and superior colliculus. Notably, rostral ECIC received a higher fraction of input from the ventral cochlear nucleus than caudal ECIC or DCIC, potentially supporting its short-latency sound responses.

Conclusions: Ongoing experiments will further compare input patterns across these IC subdivisions. These detailed connectivity data will provide new insights into the information transmitted through these IC subdivisions and guide future research to understand the functional roles of these pathways.

SU173. Effects of Fluid Volume, Viscosity, Head Orientation, and Negative Pressure on Wideband Acoustic Immittance in Middle-Ear Effusion and Residual Fluid

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Background: Otitis media with effusion (OME) is among the most prevalent conditions in the pediatric population and represents the leading cause of conductive hearing loss in children. In neonates, residual amniotic or other postnatal fluid in the middle ear can also complicate newborn hearing screening, contributing to false-positive referrals. While fluid volume and head orientation have been recognized as important factors, the combined influence of negative middle-ear pressure and the wide range of effusion viscosities (spanning $\sim 1\text{--}10,000\times$ that of water, from serous to thick mucoid) on acoustic responses remains poorly characterized.

Methods: We extended a previously validated finite-element (FE) model of middle-ear wideband acoustic immittance (WAI), tuned using simulation-based inference. Parametric sweeps examined the effects of effusion volume, head orientation, negative pressure loading, and viscosity. Baseline simulations with an air-filled cavity provided reference immittance and absorbance spectra. Comparative analyses were performed against both our own and published clinical data.

Results: Simulations showed that fluid volume and head orientation strongly altered WAI through changes in effective air space and variations of fluid–TM contact area, producing spectral shifts consistent with clinical observations. Negative pressure further accentuated impedance changes, particularly at low frequencies. Increasing viscosity reshaped the absorbance profile, introducing sharper peaks shifted above 1 kHz.

Conclusions: This study demonstrates how a fluid–structure interaction framework can explain the combined influences of testing conditions (e.g., head orientation) and pathological changes (e.g., fluid presence, volume, viscosity, and negative pressure) on WAI. By capturing these diverse conditions, the model provides mechanistic insight into how OME alters middle-ear transmission and how variability in measurement settings can shape observed WAI responses. Because WAI reflects the acoustic transmission properties of the ear, these findings also help to

interpret how middle-ear status influences otoacoustic emissions (OAEs, forward and backward transmission) and auditory brainstem responses (ABRs, forward transmission). The effect of head orientation is particularly relevant for newborns, who are typically tested in convenient, non-standardized resting positions that may increase variability, reduce test–retest reliability, and contribute to false-positive referrals. Finally, the framework establishes a foundation for applying machine-learning approaches trained on diverse fluid and orientation scenarios to improve diagnostic accuracy in pediatric OME evaluation.

SU174. Sensitivity-Guided Inference: A Structured Approach to Reduce Simulation Burden in Neural Network–Based Diagnosis

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Background: Neural networks and deep learning are increasingly used for diagnostic modeling, but their growing complexity often overshadows traditional statistical analyses essential for interpretability. Simulation-based inference (SBI) learns the mapping between model outputs and parameters and, given reference data, returns posterior distributions that can be fed into a deterministic model to reproduce the data. However, as the number of parameters increases, required simulations grow rapidly (approximately quadratically with dimensionality), slowing convergence and limiting interpretability. The middle ear, with its complex structure and many parameters, is a prime case: we propose a two-stage strategy that first ranks parameter influence via a global sensitivity procedure and then applies tiered SBI to sequentially tune high-dimensional models (from high- to lower-sensitivity subsets). This enables automated diagnosis and can inform middle-ear reconstructive surgery planning.

Methods: We propose a structured workflow that integrates systematic sensitivity analysis with multi-batch SBI to reduce simulation burden while retaining interpretability. As a case study, we implemented our approach on a lumped-element human middle-ear model that has been experimentally validated. Parameters were first ranked using one-at-a-time sweeps and global methods (Morris and Sobol total-order indices) to identify and order model parameters by their influence on system outcomes. Parameters were grouped into high-, mid-, and low-impact tiers. Multi-batch SBI was then performed iteratively: first inferring high-impact parameters while fixing others at prior baselines, then sequentially unfreezing mid- and low-impact groups. This strategy reduces dimensionality at each stage while updating priors with maximum a posteriori (MAP) estimates.

Results: Sobol ranking with Saltelli’s sampling scheme ($\approx 30,000$ simulations) produced a clear hierarchy of influential parameters. Learning-curve analysis guided the number of simulations needed for each tier, and staged multi-batch SBI yielded stable posteriors and reproducible spectra. In contrast, conventional all-parameter SBI required orders of magnitude more simulations and often failed to converge consistently or reproduce reference experimental data.

Conclusions: Sensitivity-guided, tiered inference reduces the simulation burden of SBI while preserving interpretability. The framework provides a practical workflow for parameter ranking, staged inference, and efficient posterior convergence. Applied here to the middle ear as a case study, the approach demonstrates how complex biological systems with many parameters can be analyzed in a way that identifies the properties most critical to system function while ensuring

that neural network–based diagnosis remains computationally efficient and clinically interpretable rather than a black box.

SU175. A Unified Framework for Spectral Similarity and Parameter Confidence in Neural Network–Based Diagnosis

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Background: Simulation-based inference (SBI) uses neural networks to map model parameters to outputs. This overcomes limitations of deterministic models that rely on fixed parameter sets, as SBI can capture uncertainty and variability. However, two challenges remain: (1) quantifying how well SBI-informed outputs match experimental spectra and (2) assessing confidence in inferred parameters. We propose a general framework that addresses both aspects, demonstrated here using middle-ear wideband immittance spectra.

Methods: Experimental reference data included repeated measurements of stapes velocity, ear-canal impedance, and absorbance (200 Hz–8 kHz) on a temporal bone. A finite-element model was used to generate training datasets with randomized parameter values, and SBI was trained to infer posterior distributions of seven physiologically meaningful middle-ear parameters. Spectral similarity was evaluated using three categories of measures:

- Point-to-point error metrics: MAE, RMSE, weighted RMSE, L1 and L2 norms, and maximum absolute error
- Distributional/divergence metrics: Wasserstein distance, AUC difference, and energy distance.
- Shape/trend metrics: dynamic time warping, cosine similarity, Pearson correlation coefficients.

Posterior confidence was quantified using entropy and distributional width measures (standard deviation, interquartile range (IQR), full width at half maximum (FWHM), mass width). Learning-curve analysis, varying simulation dataset size from hundreds to 10,000, identified the minimum data required for stable convergence of SBI training. Noise-sensitivity analysis evaluated the robustness and narrowness of posterior distributions under experimental variability. A majority-vote scheme integrated all metrics into two composite indices: the Spectral Similarity Index (SSI) and the Parameter Confidence Index (PCI).

Results: The SSI and PCI jointly identified conditions where SBI produced both accurate spectral fits and narrow, well-constrained parameter distributions. Learning-curve analysis revealed diminishing returns beyond a critical dataset size, while noise analysis exposed stability limits of posterior estimates. Importantly, SSI and PCI were calculated simultaneously across stapes velocity, ear-canal impedance, and absorbance, yielding a joint composite index rather than independent similarity scores for each spectrum.

Conclusions: This framework moves beyond single-metric evaluation by combining multiple similarity and confidence measures into unified indices. Although demonstrated here for model-to-data comparisons, it can be generalized to contexts where multiple spectral datasets (e.g.,

data-to-data) are compared. In doing so, it supports more robust model validation and strengthens confidence in parameter-based clinical decision-making.

SU176. Acoustic Measurement of Tympanic Membrane Displacement During Middle Ear Muscle Reflex Activation in Normal Human Ears

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Background: The same acoustic stimulus paradigm that has been used to detect middle-ear muscle reflex (MEMR) may be further analyzed to estimate the distance between the ear-canal probe and the tympanic membrane (TM). This study examines TM displacement in response to MEMR activation to infer contraction of the stapedius and/or tensor tympani muscles.

Methods: A train of acoustic clicks presented to the ear canal served as probes, and a contralateral broadband sound that increased and decreased in sound level served as a putative MEMR elicitor. Analysis of the acoustic response to click stimuli can provide meaningful interpretation when both magnitude and phase are used. Further analysis of the click response using the MEZ(Trademark) method (Merchant and Neely, 2021) provides estimates of middle-ear input impedance and TM displacement. An analog-circuit model of the ear canal and middle-ear was fit to individual click responses. The time-course of model components associated with the ossicular chain suggests how its stiffness and damping are altered by MEMR activation. The direction of TM displacement may indicate whether MEMR activation is dominated by contraction of the tensor tympani (medial) or stapedius (lateral) muscle.

Results: In the subset of measurements where (1) ear-canal impedance and middle-ear impedances were well fit by the model and (2) TM returned to its initial displacement after termination of the contralateral elicitor, TM displacement was about equally likely to be medial or lateral. Distributions of model component values across all well-fit measurements showed a significant decrease ($p < 0.01$) in stiffness of the ossicular chain and no significant change in other components.

Conclusions: Our findings are supported by a recent study of human temporal bones (Puria, 2023), which observed that contraction of the tensor tympani muscle or the stapedius muscle laterally displaces the umbo of the malleus either laterally or medially, respectively. Our approach may provide an avenue to understand the function of the tensor tympani muscle, which has remained poorly understood.

SU177. Leveraging Middle-Ear Input Impedance for Diagnosis of Conductive Pathologies

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Background: Machine learning-based classification has the potential to improve the accuracy and efficiency of diagnosing mechanical ear pathologies when applied to audiometric and wideband tympanometry (WBT) data. Previous studies show that pairing the air-bone gap (ABG) with absorbance, the most widely used WBT metric, yields better discrimination between stapes fixation (SF) and superior canal dehiscence (SCD) than either measure on its own. However, absorbance captures only magnitude and omits phase information, limiting its ability to fully characterize middle- and inner-ear mechanics. Impedance offers an advantage in this respect, but variability in ear canal acoustics may obscure its diagnostic value. By using a circuit model of the ear, these ear canal effects can be minimized, allowing the input impedance at the middle ear (ZME) to be estimated.

Methods: 97 ears were included in this study: 27 normal, 32 with confirmed SCD via CT scan, and 38 with SF confirmed via surgery. WBT data were collected with the Interacoustics Titan, and air- and bone-conduction thresholds (0.25-4 kHz) were obtained from patient audiograms. ZME was derived for each ear by fitting a lumped-element model composed of three parallel branches, each containing a stiffness (K), damping (R), and mass (M) element. The ear canal was represented as a transmission line to isolate ZME from the measured input impedance at the ear canal (ZEC). Logistic regression algorithms were trained with dimensionality reduction via PCA, and cross-validation was used to evaluate classification performance.

Results: The combination of ABGs and |ZME| achieved the lowest classification error rate (15%), outperforming ABGs and absorbance (22%). ABGs alone resulted in 20% error. Confusion matrix analyses showed that SF could be classified with perfect accuracy (100%) using ABGs and |ZME|, but SCD remained more difficult to identify, with correct rates under 70% across algorithms.

Conclusions: These findings suggest that impedance, when paired with ABGs, improves diagnostic classification compared to absorbance. Such classification algorithms could reduce reliance on imaging or surgical exploration, particularly for differentiating SF from SCD. Future work includes expanding the dataset, incorporating other conductive pathologies (e.g., ossicular discontinuity, malleus fixation), and exploring alternative model representations. In addition, the role of impedance across static pressure sweeps remains poorly understood. Future work also includes investigating whether the full pressure range from -300 daPa to +200 daPa may serve as an additional tool for distinguishing pathologies.

SU178. High-Frequency Bone-Conduction Thresholds (4–16 kHz) in Children: Feasibility, Reliability, and Early Disease Signatures

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Background: Bone-conduction (BC) hearing thresholds are rarely measured above 4 kHz in routine practice, despite well-documented examples of high-frequency (HF) conductive hearing loss. Newly characterized BC transducers (Tascam and KLH96) with high output and low distortion permit reliable HF-BC measurement (4–16 kHz) in adults. We implemented HF-BC in

pediatric otology clinic to (i) assess thresholds in normal-hearing (NH) pediatric ears and (ii) explore HF hearing loss signatures of common middle ear disease in pediatric patients.

Methods: Children 5–16 y underwent standard and HF audiometry including air-conduction (AC) and BC thresholds at 4, 6, 8, 9, 10, 11.2, 12.5, 14, and 16 kHz. Contralateral masking was used when HF-AC asymmetry was ≥ 10 dB. Reliability was tested via serial threshold measurement at select frequencies (9 and 12.5 kHz). Audiometer output was converted to force (dB re 1 μ N) and to hearing level (dB HL) using our published HF-BC calibration protocol and adult reference thresholds (18–25 y). Pediatric cohorts included NH, otitis media with effusion (OME; flat tympanogram), and post-middle-ear surgery/disease (“post-surgical”).

Results: HF-BC thresholds were feasible as early as age 5 and measurable at 4–16 kHz. Reliability was typically ≤ 5 dB at 9 and 12.5 kHz. Pediatric median force thresholds were broadly similar to adult NH subjects; but select frequencies (4, 8, 16 kHz) showed pediatric medians ~ 10 –15 dB lower (better). Thresholds obtained with the KLH96 and Tascam in the same subjects were generally similar to test-retest variability with larger differences at 4–6 kHz, indicating a possible need for device-specific reference values.

Disease signatures: In an OME case followed longitudinally, HF-AC improved after tympanostomy tube placement while HF-BC remained stable, consistent with an HF air-bone gap (ABG) due to HF conductive hearing loss. In other post-surgical ears, we observed two patterns: (i) a conductive signature (stable HF-BC with improved HF-AC), and (ii) a sensorineural signature (HF-BC thresholds elevated relative to pediatric NH medians and tracking with AC). These examples illustrate HF-BC’s ability to separate conductive from sensorineural contributions at high frequencies.

Conclusions: HF-BC audiometry (4–16 kHz) is feasible in children, reliable at 8–12.5 kHz, and clinically informative for distinguishing type of HF loss in common pediatric conditions. Pediatric force medians are largely similar to adults but may be lower by ~ 10 –15 dB at select bands; larger datasets are needed to determine whether pediatric-specific RETVFLs are warranted. Findings also motivate parallel investigations in HF-AC references in this population. Overall, HF-BC provides a practical path to quantify HF ABGs and separate conductive from sensorineural effects to guide counseling and improve surgical outcomes.

SU179. Avian Cochlear Nucleus Neurons Exhibit a Tonotopic Developmental Delay of Hcn Current

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Background: Functional hearing relies on the precise frequency-specific encoding of auditory stimuli. Nuclei in the auditory brainstem, such as the mammalian cochlear nucleus and the analogous avian nucleus magnocellularis (NM), are organized by different frequency-specific regions known as tonotopy. HCN channels across different frequency regions help support tonotopic tuning by the underlying mediated current known as I_h . While it is known that low-frequency regions of NM exhibit a developmental delay compared to the high-frequency areas,

differences in HCN channel expression and I_h across development and tonotopic regions remain unknown. This study aims to fill this knowledge gap.

Methods: We recorded from high- and low-frequency regions (HF and LF, respectively) of brainstem slices before (embryonic (E) days 12-13), during (E15-16), and after hearing onset (E20-21). Voltage clamp recordings from NM neurons measured I_h from holding voltages between -40mV to -130mV in 10mV increments. I_h was measured with Steady State (SS) and different current techniques and compared. Fast and slow I_h activation kinetics were fit with single and double exponentials to explore HCN channel subunit contribution.

Results: I_h increases significantly across development for all NM neurons. However, in embryos before hearing onset, high-frequency neurons have significantly more I_h than low-frequency neurons. In embryos during hearing onset, HF and LF difference currents are similar, while HF neurons still have a greater inward SS current than LF neurons. Finally, for embryos after hearing onset, there is no significant difference between HF and LF neurons for either SS or difference current. Activation kinetics are similar across the tonotopic axis throughout development.

Conclusions: Results suggest that at the beginning of neural circuit development, HF neurons have more HCN channels than LF neurons. Furthermore, I_h magnitudes increase across development with HF neurons developing sooner than LF neurons with respect to HCN channels. Since activation kinetics do not differ across development or tonotopy, results suggest that subunit composition of HCN channels is not changing during development, nor is it different across the tonotopic axis. Taken together, we conclude that LF neurons experience a developmental delay in the number of HCN channels present, with I_h catching up to HF neurons by the onset of hearing. Characterizing the development of auditory brainstem neurons across the tonotopic axis is a crucial step in elucidating how normal central auditory systems function effectively in vertebrates.

SU180. Auditory-Visual Speech Integration is Supported by Frontal-Cortical Engagement in Older Adults

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Background: Older adults with age-related auditory (AO) and visual (VO) speech perception deficits can often identify audiovisual (AV) speech to a degree of accuracy like that of younger adults. We have previously reported that this enhanced AV speech perception (relative to unisensory deficits) by older adults can be explained by auditory-visual multisensory perceptual benefits that are > in younger adults. Poorly understood, however, are the neural mechanisms that support AV speech perception in older adults with age-related auditory and visual deficits. Cortical activity in inferior-frontal and temporoparietal cortex has previously been found to be a stronger predictor of AV speech encoding in older adults than younger adults, especially in noisy listening conditions, suggesting that higher-level cortical engagement may support auditory-visual speech integration in older adults. Here we examined how the functional connectivity between inferior-frontal cortex and temporoparietal cortex can predict auditory-visual enhancement of AV speech perception in younger and older adults. We hypothesized that

stronger functional connectivity between cortical areas would predict more auditory-visual enhancement in older adults than in younger.

Methods: A group of 10 younger normal-hearing adults (20-30 years of age, 7 female) and 14 older adults (54-80 years of age, 7 female) with normal hearing or mild-to-moderate sensorineural hearing loss completed an audiovisual speech identification task, identifying words spoken in Gaussian noise (-5, 0, +5 dB SNR) presented in AO, VO, and AV conditions. We calculated auditory-visual enhancement from identification performance across conditions [$AVE=AV-(AO+VO)$]. Speech-evoked potentials (SEPs) were recorded separately while participants were passively presented consonant-vowel syllables (/ba/, /da/, /ga/) in audiovisual conditions in silence and in Gaussian noise at 0 dB SNR (240 trials per condition). Each participant's AV SEP and T1 structural image were used to source-constrain their SEPs to inferior-frontal cortex and temporoparietal cortex to measure the response generated in each cortical area. For each participant, metrics of functional connectivity between inferior-frontal and temporoparietal cortex were computed for responses evoked by AV speech (cross-correlations and dynamic time warping distance).

Results: AV SEP response latencies in inferior-frontal and temporoparietal cortex were longer for older adults than younger adults in inferior-frontal and temporoparietal cortex. AV response latencies in inferior-frontal cortex were shorter in noisy listening conditions, but only for older adults. Importantly, greater functional connectivity between inferior-frontal and temporoparietal cortex when listening to AV speech in noise predicted larger multisensory benefits in speech identification (AVE).

Conclusions: These preliminary findings suggest that older adults rely on a functional network between inferior-frontal and temporoparietal cortex when identifying AV speech in noisy listening conditions, more so than younger adults. The results will be discussed with regards to age-related changes in sensory processing and cognition and the potential role of visual input in alleviating age-related deficits in auditory speech communication.

SU181. Mechanisms for Generation and Control of EMREOs (Eye Movement-Related Eardrum Oscillations)

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Background: Accurate information about eye movements is necessary for audiovisual integration because such movements shift the positions of the eyes with respect to the head/ears. We have recently reported an eardrum oscillation, time-locked with eye movement onset and occurring in the absence of outside auditory stimuli, that suggests eye movement information is sent by the brain to the ears (Eye movement-related eardrum oscillation, EMREO; Gruters et al. PNAS 2018; Lovich et al. PNAS 2023). But what is the proximal and neural mechanism producing such eardrum oscillations? The chief suspects are the middle ear muscles (MEMs) and the outer hair cells (OHCs).

Methods: We recorded eye movements and associated eardrum oscillations in rhesus monkeys before and after surgical intervention involving the MEMs -- specifically, stapedotomy and

tensor tympani tenotomy. Such transections of the tendons connecting the MEMs to the ossicles are typically done in human patients suffering from otosclerosis or MEM myoclonus, and the procedures were adapted here for the monkey. The procedures served to eliminate the contribution of the particular MEM in question to the EMREO.

Results: Sectioning MEM tendons did not eliminate the EMREO. Following stapedotomy, the EMREO was reduced in amplitude but still occurred. Following tensor tympani tenotomy, the EMREO was actually increased in amplitude. When stapedotomy and tensor tympani tenotomy were combined, the EMREO still occurred, and was again increased in amplitude. Together these findings suggest the MEMs are not the sole cause of the EMREO, but rather regulate its amplitude likely in a push-pull fashion. These results from animals are largely consistent with related preliminary data in human clinical patients who have undergone similar procedures.

A general caveat to the interpretation of the MEM surgical interventions is that the procedure may change the mechanical load on the eardrum such that interpretations of changes in amplitude of the EMREO signal should be confirmed by additional research. However, that the EMREO continued to exist even after both MEMs were disconnected from the ossicles indicates that there must be an additional source of force contributing to the signal. The most likely such source is the OHC, which could potentially generate this signal by oscillating in concert throughout the cochlea. It is also possible that in the event of OHC dysfunction, the signal can be generated by MEMs acting alone, a possibility not tested here.

Conclusions: We interpret these findings in the context of how eye movements may alter sound localization cues. Recent work has shown that forces applied by the MEMs to the eardrum in a cadaver model could alter both interaural timing and level difference cues in an eye movement-dependent way (Cho et al., Hearing Research 2023).

SU182. Component Analysis of Distortion Product Otoacoustic Emissions in Children for f2 up to 12 kHz

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Background: Distortion product otoacoustic emissions (DPOAE) recorded in the ear canal are mixtures of non-linear distortion and linear reflection components. Each component has its own hypothesized generation source and mechanism based on its phase-frequency behavior. However, little is known about the components' behavior in the first decade of life particularly for $f_2 > 8$ kHz. Here we report on individual DPOAE components measured using low stimulus levels, which are more sensitive to cochlear status yet difficult to measure in noisy ears. Thus, multiple signal cleaning strategies for handling noisy recordings are described.

Methods: 94 children between the age of 6 weeks and 11 years were recruited from a maternity hospital in São Paulo, Brasil. With the Etymotic Research 10X system, DPOAE were measured using fixed ratio of 1.22 for f_2 frequencies between 1.5 and 12 kHz and $L1/L2 = 55/40$ dB FPL using a 96 kHz sampling rate. Time domain waveforms were sent through a multi-step artifact rejection protocol followed by a least-squares-fit based frequency analysis and weighted

averaging procedure. The complex level and phase were then processed using continuous wavelet transform, during which the complex signals were convolved with an array of mother wavelet basis functions and resulting coefficients were filtered into different latency bands according to Moleti et al (2008). The filtered signals were separated as zero-latency distortion components and non-zero-latency reflection components. The time-frequency representations as well as level and phase behaviors were compared for three signal cleaning methods.

Results: 76 ears had recordable DPOAE above the noise floor at primary levels of 55/40 dB FPL. Nine ears with the highest quality recordings as determined by 9 dB SNR criteria across 90% of the spectrum were used as the gold standard for our analysis. The time-frequency representations of these nine ears demonstrated DPOAE energy hotspots that were constrained in the first and second latency bands with little to no signal outside of these bands. In contrast to these nine exemplar recordings, the time-frequency representations of noisier recordings displayed energy at latencies outside of the expected frequency-latency function at randomly distributed spectral regions. Furthermore, phase behavior of the total DPOAE and distortion component occasionally varied with frequency, a phenomenon not consistent with the fixed phase of distortion emissions in fixed-ratio recordings.

Conclusions: The influence of physiological and environmental noise on DPOAEs evoked by low stimulus levels must be considered when separating individual components. Advanced signal processing methods such as SNR-weighted averaging, multi-step artifact rejection, continuous wavelet transform that can numerically quantify the unwanted noise residuals, and time-frequency representations that can visually display noise contributions may be useful and will be discussed. Future studies are warranted to examine the utility of these methods for improving detection of DPOAE responses at low stimulus levels.

SU183. A Transmission Line Model of the Cochlea Under Noise Produces Four Distinct Categories of Spontaneous Emissions

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Background: Spontaneous Otoacoustic Emissions (SOAEs) are indicative that cochlea is an active, nonlinear system. Existing research has shown the transmission line models of the cochlea with roughness can predict narrow band peaks in the absence of any external stimulus, i.e. SOAEs; however, these models are limited to only describing narrowband bimodal peaks. Existing human SOAE experimental data show that SOAEs include not only narrowband bimodal peaks but also broadband unimodal peaks.

Methods: The cochlea is studied using a modified version of the Ku and Elliot model, which is a 1D transmission line model. Roughness is introduced by adding small (0.25%) perturbations to the activity parameter γ . Full spectrum white noise is applied to the basilar membrane and is uncorrelated in time and space. The nonlinear dynamics of the model are simulated numerically in the time-domain before analyzing the spectrum of the response at the stapes and evaluating how the key characteristics of the spectrum are linked to the linear stability analysis of the model. Spectral peaks are characterized by their quality factor (tuning sharpness), level, and their time domain distribution.

Results: The model with noise predicts a series of spectral peaks with a wide range of levels, quality factors, and Hartigan dip statistic values. Comparison of the SOAE spectrum to the linear stability diagram (eigenvalues of the state-space matrix) helps to interpret the numerical results. Our analysis suggests that SOAE peaks can be naturally divided into four categories: (1) Primary SOAE Type A peaks; (2) Primary SOAE Type B peaks; (3) Filtered noise peaks; (4) and Distortion product SOAE (DPSOAE) peaks. Primary SOAE Type A and B peaks are associated with unstable eigenvalues; Type A appear in the noiseless case and the case with noise, and Type B appear only in the case with noise. Filtered noise peaks are associated with nearly unstable eigenvalues and act as lightly damped harmonic oscillators. Distortion product SOAE peaks are peaks which are distortion products of primary SOAE peaks.

Conclusions: With an extension to the existing linear stability analysis and the introduction of noise into the transmission line model of the cochlea, we demonstrate how the shortcomings of existing transmission line models can be overcome, and we observe additional phenomena from the introduction of noise which was not previously predicted. The presence of different categories of SOAE peaks provides a novel lens through which to understand SOAE spectra through.

SU184. Growth of DPOAEs Evoked by the Fixed L1 Stimulus Paradigm

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Background: Distortion product otoacoustic emission (DPOAE) magnitudes measured across a range of stimulus levels as level growth functions (LGFs) may be useful for assessing cochlear function and health. The observed LGF shapes depend on the variety of stimulus paradigms that have been reported in the literature. Recent work has suggested that the slopes of LGFs from the fixed L1 paradigm may differentiate types of cochlear hearing loss; however, normal LGF growth in this paradigm is not well understood. LGFs are typically fitted with mathematical functions from which slopes can be derived and (behavioral) thresholds can be predicted. However, fitting LGFs at low stimulus levels is challenging, due to poor signal-to-noise ratios (SNRs). Here, we describe a generalized linear statistical model (GLM) appropriate for fitting LGFs at varying SNRs, including portions of the LGF that fall below the noise floor. The primary aim of this study was to characterize fixed L1 LGFs at low-to-moderate levels in young listeners. A secondary aim was to evaluate the hypothesis that functions fit to these LGFs accurately predict DPOAE magnitude at low stimulus levels ($L2 = 5$ dB FPL), which produce responses that are typically below measured noise floors.

Methods: DPOAE LGFs were recorded from 12 young, healthy adult participants (9 female, mean = 22.9 yr) using an ER10X probe system and custom software. L1 was held constant at 65 dB FPL, while L2 swept in level from 0 to 70 dB FPL in 9 seconds. Each test consisted of 24 nine-second sweeps for a total test time of 216 seconds. For evaluating the fit at low levels, discrete measurement was also obtained at $L2 = 5$ dB FPL, a low stimulus level eliciting DPOAEs well below the typical noise floor. This low-level DPOAE was recorded for a long period of time (~26 minutes averaging time) to reduce the noise floor to near -40 dB SPL. A phenomenological model was used to generate simulated data for comparison with human data.

Results: Data from healthy young adult listeners, along with simulations from a phenomenological model, established that at low to moderate L2 and a fixed L1, LGFs grow as polynomials dominated by the linear term, with smaller but important contributions from the quadratic and cubic terms. The GLM predicted DPOAE magnitudes at low stimulus levels with a mean error < 0.4 dB (SD = 2.2 dB).

Conclusions: This improved understanding of expected LGFs in healthy ears provides a foundation for future studies of the relationship between fixed L1 LGFs and cochlear health.

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SU185. Auditory Novelty Detection Impairment in Rats Treated With the Nmda Receptor Antagonist MK-801: A Model of Schizophrenia

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Background: Animals are constantly exposed to a wide range of environmental sounds. While many of these are irrelevant, the detection of novel stimuli is crucial for survival. Behavioral habituation, a fundamental form of learning, is characterized by the progressive reduction of responses to repeated stimuli and provides the foundation for detecting unexpected changes (Rakin, 2009; Netser et al., 2011; Quintela-Vega et al., 2023). This ability is closely linked to mismatch responses observed at both neuronal and behavioral levels, which have been conceptualized within the predictive coding framework. These responses rely on repetition suppression to predictable stimuli and prediction error signals when expectations are violated. Importantly, reduced mismatch responses have been consistently associated with schizophrenia and glutamatergic dysfunction in humans. To investigate this, we used a pharmacological model based on the NMDA receptor antagonist MK-801, which reproduces schizophrenia-like features by disrupting neuronal adaptation and associative learning mechanisms (Parras et al., 2017; Ishishita et al., 2019).

Methods: We trained eight freely moving female Long Evans rats to discriminate deviant tones in an oddball paradigm. Animals learned to nose-poke in response to deviants, which were rewarded with food pellets. A pair of frequencies within the auditory range (8.0–11.3 kHz) was used, and discrimination was tested across inter-stimulus intervals (1.0, 1.5, and 2.0 s) with a 10% deviant probability. A many-deviant paradigm was also applied to control for predictive coding indices: mismatch (iMM), prediction error (iPE), and repetition suppression (iRS). Behavioral performance was quantified using d' index. Once the animals reached reliable performance (d' > 1), they were divided into two groups: control and MK-801. Both saline and MK-801 (0.075 mg/kg) were administered subcutaneously 45 min before the behavioral deviance detection task. Finally, neuronal activity was recorded in the auditory cortex and the inferior colliculus using multi-channel probes and equivalent paradigms to study neuronal responses.

Results: Preliminary results show that saline-treated animals continued to improve in the deviance detection task during treatment, whereas MK-801-treated rats exhibited a significant impairment in performance after drug administration.

Conclusions: These findings suggest that NMDA receptor blockade with MK-801 disrupts auditory novelty detection at both behavioral and neural levels. This pharmacological model provides valuable insights into the mechanisms underlying sensory and cognitive deficits in schizophrenia.

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SU186. Serotonergic Modulation of Auditory Cortical Circuits

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Background: Auditory experience powerfully shapes the functional organization of the developing auditory cortex (ACtx), but this plasticity declines in adulthood. Neuromodulator release can reinstate robust plasticity in the adult brain, offering potential to promote recovery from neurological disorders or peripheral hearing loss. However, the mechanisms by which distinct neuromodulators act within cortical circuits to drive plasticity remain incompletely understood. For example, serotonin (5-HT) modulates tonotopic plasticity in the adult ACtx, but the underlying cellular and circuit mechanisms are unknown. Prior studies, including our own, have mapped serotonergic axons throughout ACtx and localized diverse 5-HT receptor mRNAs to distinct populations of excitatory pyramidal neurons and GABAergic interneurons. Specifically, subsets of deep-layer somatostatin-expressing (SST) inhibitory interneurons predominantly express transcripts encoding inhibitory 5HT1A receptors and may therefore serve as key targets of serotonergic modulation. We hypothesize that 5-HT selectively suppresses SST inhibitory interneurons to dynamically sculpt network activity.

Methods: To identify the effects of 5-HT on SST interneurons, we combined whole-cell electrophysiology and two-photon (2-P) imaging in thalamocortical slices of mouse ACtx.

Results: Local 5-HT application reliably hyperpolarized layer 5 (L5) SST interneurons and suppressed spike amplitude, which was abolished by bath application of a selective 5-HT1A antagonist. Given that subsets of SST interneurons expressing the 5-HT1A receptor form local projections, we employed optogenetic activation of SST interneurons to probe serotonergic modulation of SST inhibitory output onto L5 pyramidal neurons. We observed that bath-applied 5-HT reduced SST-mediated inhibition onto the majority of these neurons.

SST interneurons have been previously shown to curtail runaway excitation during periods of prolonged cortical activity. Using 2-P imaging to interrogate the effects of 5-HT on the cortical network, we demonstrated that 5-HT prolongs calcium responses evoked by repetitive thalamic

stimulation in subsets of L5 pyramidal neurons, consistent with reduced SST-mediated inhibition.

Conclusions: Ongoing work is examining whether serotonergic actions on specific SST interneuron subclasses mediate layer-specific modulation. L5 serves as the principal output layer of the cortex, suggesting that 5-HT may gate information flow from primary ACtx to higher-order cortical and subcortical targets. Together, these studies provide new insight into the cellular and circuit mechanisms by which 5-HT modulates ACtx, informing the development of targeted strategies to induce layer-specific neuronal changes that may enhance adult cortical plasticity.

SU187. Decision-Making Shapes Auditory Cortical Responses to Harmonic Complex Tones in Noise

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Background: Background noise often presents a challenging acoustic environment for listening. Adaptive plasticity in auditory cortex is thought to facilitate hearing in noise by adjusting the gain of neural responses to behaviorally meaningful sounds. However, it remains unclear how adaptive plasticity facilitates the recognition of auditory perceptual features with masked acoustic components, such as the missing fundamental frequency (F0) of a harmonic complex tone (HCT) in background noise. Since mice have high-frequency hearing, neurons in auditory cortex that are tuned to low F0s encode missing-F0s in HCTs. Thus, we hypothesized that adaptive plasticity in mouse auditory cortex facilitates the recognition of missing-F0s in noise by modulating the activity of F0-selective neurons relative to background noise level.

Methods: Background noise often presents a challenging acoustic environment for listening. Adaptive plasticity in auditory cortex is thought to facilitate hearing in noise by adjusting the gain of neural responses to behaviorally meaningful sounds. However, it remains unclear how adaptive plasticity facilitates the recognition of auditory perceptual features with masked acoustic components, such as the missing fundamental frequency (F0) of a harmonic complex tone (HCT) in background noise. Since mice have high-frequency hearing, neurons in auditory cortex that are tuned to low F0s encode missing-F0s in HCTs. Thus, we hypothesized that adaptive plasticity in mouse auditory cortex facilitates the recognition of missing-F0s in noise by modulating the activity of F0-selective neurons relative to background noise level.

Results: Mice successfully discriminated between target vs. non-target F0s in silence ($d' > 1$), with performance declining as SNR decreased. Average neural responses to HCTs also declined with SNR, however, neural responses depended strongly on behavioral choice. As SNR decreased, neural responses during behavioral hits and false alarms increased and decreased, respectively. In contrast, neural responses during correct rejections and misses were less choice-dependent, generally increasing with noise level.

Conclusions: Our results suggest that during perceptual discrimination of missing-F0s, neural responses to HCTs in noise reflect a combination of sensory coding and decision-making processes in auditory cortex. Together, these mechanisms may facilitate listening in challenging acoustic environments.

SU188. Maturational Changes in Listening Effort During a Degraded Speech Task in Adolescents and Young Adults

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Background: Understanding speech in challenging listening conditions, such as noisy environments or with degraded signals, is a skill that requires intact auditory encoding, deployment of listening effort, and application of cognitive skills. While some studies have indicated that perception of degraded speech continues to mature during adolescence, others have not. Here we examined (1) whether behavioral performance on a degraded speech task changes during adolescence and young adulthood, (2) how adolescents and young adults deploy listening effort during this task, and (3) the relationship between task performance and listening effort over this age range.

Methods: We tested 10- to 23-year-olds (n=76) on 6-band noise-vocoded (NV) speech under two conditions: NV speech immediately followed by a period of silence or NV speech immediately followed by multi-talker babble. Listeners were asked to repeat back what they heard following either the silent period or the babble. Based on Winn and Moore's (2018) prior work with hearing-impaired listeners, we hypothesized that it would require more listening effort for all listeners to comprehend the speech when it was followed by babble, but that these effects would be more pronounced in adolescents. We assessed listening effort using an eyetracker to measure pupil size during pre-trial baseline, the sentence, the babble or silence, and the participant's response (i.e., repeating back the sentence). We analyzed pupil size change relative to baseline over the course of the trial and compared these temporal dynamics between the silence and babble conditions and between the age groups. Consistent with convention in the field, larger pupil size compared to baseline was interpreted as indicative of greater listening effort.

Results: The temporal dynamics of listening effort differed between the age groups and the two listening conditions. In addition, the adolescents demonstrated greater listening effort despite poorer performance on the degraded speech comprehension task.

Conclusions: These data suggest that degraded speech perception continues to mature during adolescence. Poorer performance by teenagers occurred despite higher levels of listening effort, indicating that their prolonged perceptual development is not attributable to poor engagement. Other factors that may contribute to this late maturation include developmental changes in auditory encoding and cognitive processing.

SU189. Ideal Observer Analysis of the Effects of Medial Olivocochlear Gain Control on Neural Coding of Sound Level and Amplitude Modulation

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Background: The medial olivocochlear (MOC) system reduces cochlear gain in response to sound. One hypothesis for the MOC system's role is that it adjusts cochlear gain to improve

sound coding at moderate sound levels. For example, when detecting amplitude modulation (AM) of a mid-level signal, a reduction in cochlear gain could reduce the deleterious effects of cochlear compression and/or synaptic saturation and enhance AM detection. This gain reduction could be elicited by sound preceding the AM stimulus (precursor). Some psychophysical data on level discrimination and AM detection with precursors appear qualitatively consistent with this hypothesized role of the MOC system. However, due to nonlinearities present in the auditory periphery, it is difficult to predict how much cochlear gain changes should be expected to change behavioral thresholds.

Methods: To quantitatively predict the effects of MOC gain control on level discrimination and AM detection, we used a model of the auditory periphery and midbrain modified to include a simple MOC reflex driven by stimulus energy. We simulated responses in this model to level discrimination and AM detection stimuli with and without precursors and then used ideal observer analysis to determine theoretical bounds on the effects of cochlear gain changes on task performance. Both rate-based and temporal coding schemes were evaluated, and we applied the framework to several stages in the model to better understand how the effects of MOC gain control manifest along the ascending auditory pathway.

Results: Ideal observer analysis revealed that, at moderate sound levels in single model channels, reductions in cochlear gain enhance level discrimination and AM detection, consistent with classical theories of MOC gain control. However, effect sizes for level discrimination and AM detection were small in peripheral model stages for both rate-based and temporal codes. For example, at 60 dB SPL in low-spontaneous-rate auditory-nerve fibers, a 10-dB change in cochlear gain resulted in a predicted change in level discrimination thresholds of only ~1 dB and AM detection thresholds of only ~2 dB, raising questions about whether these effects would be detectable behaviorally. Moreover, in contrast to single-channel results, when observers had access to off-frequency channels, predicted effects of cochlear gain on perceptual sensitivity were negligible.

Conclusions: Our simulations support the idea that the MOC system enhances coding of sound level and AM. However, at the same time, our simulations suggest that surprisingly large changes in cochlear gain may be required to produce measurable threshold changes in behavioral tasks designed to explore the MOC reflex.

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SU190. Measures of Subclinical Hearing Function: Long-Term Test-Retest Reliability and Relations Between Change Outcomes

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Background: Noise exposure is the main cause of preventable hearing loss (Rabinowitz, 2000) and recreational noise may pose a particular hazard in young people (WHO, 2015). Studies of hearing health in the younger population (e.g., Henderson et al., 2011; Carter, 2014; Keppler et al., 2015) have tended to focus on pure-tone audiometry (PTA: detection thresholds of pure tones

at different frequencies) but PTA may be relatively insensitive to the early effects of noise damage.

We conducted a longitudinal study investigating changes in auditory function over a three-year period, during which many participants started regularly attending high-sound-level environments (e.g., live music events and nightclubs). Our participants underwent an extensive battery of physiological, psychoacoustic and self-report measures with the aim of identifying early markers of noise damage. Here we report on relations between the various hearing change measures, with particular focus on disentangling the effects of hair-cell damage and synaptic pathology. We also report on the longer-term test-retest reliability of potential early markers of recreational noise damage in young people.

Methods: Participants were 220 teenagers aged 16 or 17 at the time of their baseline test session (Nov 2021 – Dec 2022). Follow-up test sessions took place from Nov 2024 to Jan 2026. At each test session, participants completed a battery of hearing tests:

- Cochlear function: Pure-tone audiometry (0.25-8 kHz), extended high-frequency audiometry (1/3-octave narrowband noise centred at 10 kHz, 12.7 kHz, and 16 kHz), and distortion-product otoacoustic emissions (2f1-f2 amplitude, at f2 frequencies of 3-16 kHz).
- Auditory neural function: Auditory brainstem responses to monaural filtered chirps and ipsilateral middle-ear muscle reflexes with two tonal probes (226 Hz and 1 kHz) and a 4-kHz tonal elicitor.
- Speech perception: Three digits-in-noise tests (monaural digits in speech-shaped noise, diotic digits in spatially separated babble, and diotic digits with two competing, concurrent, spatially separated digit streams).
- Self-report measures: short-lasting and long-lasting spontaneous tinnitus.

Hearing change measures were defined as the difference or the ratio between these outcomes measured at baseline and then again at follow-up (post – pre or post/pre).

Results: At the time of abstract submission, 180 of the 220 participants had been invited to complete the final testing session, of which 170 had attended. The study was powered based on 60% retention, yet participant retention was much higher: on track for ~89% (n = 195) by the close of the study. Data analysis was scheduled for the fourth quarter of 2025.

Conclusions: The large sample size, longitudinal design, and broad array of measures offer novel opportunities to explore the reliability and interrelations of subclinical hearing measures over the course of years. Results will have implications for the interpretation of research reliant on the use of such measures in young adults.

SU191. Identifying the Acoustic Fingerprints of Trigger Sounds and Predicting Discomfort for Misophonia

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Background: Human hearing is critical to everyday communication and the perception of natural auditory scenes. For individuals with misophonia, sounds commonly experienced in daily life can evoke severe discomfort and distress. Aversion is often described in terms of broad

sound categories, such as bodily sounds, but what acoustic features cause specific sounds to be aversive or not, within the same category or across different individuals, remains unclear. Here, we explore whether bottom-up statistical sound features processed in the auditory periphery and midbrain can explain aversion to sounds.

Methods: Using the Free Open-Access Misophonia Stimuli (FOAMS) dataset and a hierarchical model of the auditory system, we find that sound summary statistics extracted from the auditory model can predict discomfort ratings in participants with and without misophonia. The model was fit to each individual using Generalized Perceptual Regression (Clonan et al 2025) which produces individualized transfer functions that pinpoint specific spectrotemporal modulations that contribute towards sound aversion. Model parameters were also compared to diagnostic evaluations of misophonia (Duke Misophonia and S-Five questionnaires).

Results: Overall, the model explains 76% of the variance in discomfort ratings, and we find substantial differences across participants in which sound features drive aversion. An advantage of the approach here is that it is sound-computable – perceptual ratings can be fit from or predicted for any sound. To illustrate applications of sound-computable models, we consider 1) extrapolation to a large set of untested environmental sounds and 2) personalized trigger detection in continuous audio. Model predictions accurately predict aversion to FOAMS sounds and identify untested sound categories, not in the original FOAMS set, that may also be aversive. This suggests that there may be substantial heterogeneity in how aversive specific sounds are within some sound categories. In continuous audio, we show how sound-computable models can identify the timing of potential triggers from sound mixtures. Finally, parameters of the model that account for the listener’s perceptual sensitivity were significantly correlated with diagnostic evaluation scores.

Conclusions: Altogether, our results suggest that acoustic features – spectrotemporal modulations, in particular – can practically be used to characterize the individualized patterns of aversion in participants with misophonia. Future perceptual studies using synthetic sounds and sound sets with more diverse acoustics will allow model predictions to be tested more broadly; however, sound-computable models may already have applications in precision diagnosis and management of misophonia. Funded by NIH (R01DC020097).

SU192. The Contribution of Speech Articulatory and Statistical Sound Features Towards Speech Recognition in Natural Auditory Scenes

Alex Clonan^{*1}, Ian Stevenson¹, Monty Escabi¹

¹*University of Connecticut*

Background: Humans excel at recognizing speech in natural auditory scenes despite large variation in speakers’ voice quality, intonation, and pitch. We previously demonstrated that different types of background sounds and their spectrotemporal statistics can improve or worsen speech recognition accuracy (Clonan et al 2025). However, how foreground speech features, such as fundamental frequency or formants, influence speech recognition accuracy is not well understood.

Methods: Here we explore how different speech articulatory features interact with natural and perturbed background sounds to influence speech recognition abilities. Participants (n=10) were required to recognize digit sequences (e.g., 0-9-1, 2-5-7) generated by adult and adolescent male

and female talkers and then mixed with various natural background sounds (e.g., water, babble, construction noise etc.; SNR= -9 dB) and perturbed background variants with distorted spectrum or modulation statistics. To characterize the unique interactions between the foreground and background sound statistical features, we used a hierarchical model of the auditory periphery and auditory midbrain, paired with generalized perceptual regression (GPR; Clonan et al 2025). GPR models perceptual judgments of single listeners with the neural-acoustic model and allows perceptual outcomes for arbitrary speech-foreground combinations to be predicted using auditory model features.

Results: We found that different spoken digits had different accuracies depending on both the background sound statistics and the speaker's voice. To explore these speech driven effects, we compared recognition abilities across the speech fundamental (F0), first formant (F1), and second formant (F2) frequencies, and harmonicity. Using a generalized linear mixed effects model, we found substantial, statistically significant trends for digit identity, F0, F1, and F2, on recognition accuracy, in addition to background-driven effects. Furthermore, we find that the neural inspired GPR model captures nearly 76% of the perceptual variance across background and digits. This model, based on spectral and temporal modulation statistics, successfully explains differences in recognition accuracy associated with individual talkers and talker identity (male, female, adult, adolescent) as well as differences associated with formant and harmonicity structure.

Conclusions: These results further identify foreground and background sound features that influence speech recognition in natural environmental noise and suggest that auditory computations involving modulations may underlie perceptual outcomes. Funded by NIH (R01DC020097).

SU193. Similarity and Divergence in Human and Machine Speech Recognition

Annika Magaro¹, Erica Shook², Alex Kell¹, Gasser Elbanna³, Mark Saddler⁴, Josh McDermott¹

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Background: Advances in machine learning have yielded machine systems that often approach human levels of performance on real-world perception tasks. For instance, models trained to recognize speech in noise reproduce some characteristics of human speech recognition. However, human and model speech behavior have not been extensively compared, leaving the extent to which such models can account for human speech perception unclear.

Methods: We measured word recognition abilities in humans and models across 37 types of speech distortions, spanning natural sources of distortion (e.g. background noise and reverberation) and artificial distortions (e.g. noise vocoding and audio filtering). We evaluated a set of industry speech recognition models along with in-house models. To investigate the factors that might give rise to human-like performance, we manipulated the architecture and the variability of training data for the in-house models. We measured the match between human and model performance across distortions.

Results: Two recent industry speech recognition models trained on hundreds of years of labeled speech (Whisper and Parakeet) exhibited high rank correlations with the pattern of human performance, but in absolute terms were superhuman on most types of distortion, and as a consequence were poor quantitative models of human speech recognition. In-house models with transformer-based architectures trained on a dataset that was intended to be more realistic in size (around 1400 hours) and variability produced better quantitative matches to humans. The match to human behavior was worse for models trained on less variable speech data, and for models that had exclusively convolutional architectures, even when they were matched in number of parameters.

Conclusions: The results are consistent with the idea that the robustness of human speech recognition results from the variability of real-world experience, but suggest that the computational architecture contributes to obtaining human-like generalization. Overall, the results show that superhuman performance is possible with systems that are optimized over enough data, and indicate that accurate models of human speech recognition can be obtained by optimizing models over the amounts and types of speech audio that humans are plausibly exposed to over developmental timescales.

SU194. Benchmarking Artificial Neural Network Models of Human Auditory Processing With Sound Similarity Judgements

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Background: With the increasing use of artificial neural networks (ANNs) in psychology and neuroscience, it is critical to pick appropriate benchmarks to assess how well such networks capture aspects of human perception and cognition. Most of this development has occurred in the visual domain, thus the generalizability of these visual model benchmarks to other sensory domains remains unclear. Here, we explored one possible benchmark: the extent to which human similarity judgments could be used to predict model-brain alignment across a broad range of ANNs trained on auditory tasks.

Methods: From a set of 20 ANNs, we computed cosine similarity scores between model activations for pairs of sounds. Sounds came from a dataset we compiled to be representative of sounds encountered in daily life. Models varied in architecture, training data, and training task, however none were trained on a similarity task. We calculated Spearman correlation between each models' similarity scores and sound pair similarity judgements from human participants. Additionally, we compared these correlations to a neural model-brain alignment metric from fMRI data to investigate whether models that best captured human similarity judgments also explained the most variance in neural data.

Results: The strength of correlation between similarity metrics computed from ANNs and human similarity judgements varied across models, though some ANNs did approach the human-to-human correlation ceiling. In general, ANNs trained on multiple tasks or trained on audio event categories had higher correlations to human similarity measures. Furthermore, the extent to which a given ANN captured human-like similarity judgements was strongly correlated with how well the ANN captured neural firing in its layer activations, but there was some unique variability in each measure.

Conclusions: The correlation between model similarity structure and human similarity judgments offers a task-agnostic benchmark for evaluating brain-model alignment. The variability across different models suggests that the metric has sensitivity to distinguish different models. Much like networks trained on vision tasks, some auditory ANNs captured human similarity judgements to a high degree, suggesting that these models share some auditory representational features with humans. Brain-model alignment measured with this similarity benchmark was positively correlated with a model's ability to capture neural firing patterns in its activations; therefore, this benchmark could potentially be used as an exploratory precursor for cortical brain-model alignment studies.

SU195. Hearing-In-Noise Problems Within a Large Online-Recruited Cohort

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Background: The primary clinical measure for hearing loss is the audiogram. However, suprathreshold hearing-in-noise problems only correlate moderately with absolute hearing thresholds. Consequently, audiometric thresholds fail to capture enough information for an individual's hearing performance characterization (Kollmeier, 1999, *Psychophysics, physiology and models of hearing*, pp. 211-218). There is even a considerable percentage of the population that has hearing-in-noise problems despite clinically normal audiometric thresholds, loosely categorized by terms such as "hidden hearing loss" or "central auditory processing disorder" (Schaette and McAlpine, 2011, *J Neurosci.*, 31(38), pp. 13452-7; Tremblay et al., 2015, *Ear and hearing*, 36(6), e290-e299). We ran a lab study measuring audiometric thresholds as well as hearing-in-noise to characterize an individual's hearing to find emerging measurements or model parameters for hearing characterization. In a further step, we introduced remote testing with a large participant cohort (N = 627, 4 different languages) measuring the same hearing-in-noise tests as in the lab experiments to enlarge sample size and to meaningfully identify types or principal components of suprathreshold hearing and to evaluate online testing procedures in a broader test battery.

Methods: Audiometric thresholds, loudness growth functions and tone- as well as speech-in-noise thresholds in 50 participants with age-typical or mild hearing loss who had no hearing aid experience were measured in the lab. 24 participants from the lab study also participated in remote testing to form a validation group to compare lab and remote test results. Moreover, 627 participants took part in remote testing.

Results: Correlations and RMS error-analysis revealed tone-in-notched-noise- and N0S π thresholds as potential candidates to characterize an individual's hearing by predicting numerous tone- and speech-in-noise measurements with high accuracy in the lab study. A single 500-Hz N0S π threshold explains 61% of the variance in diotic speech-in-noise performance (RMS error = 0.80 dB). Including remote test results revealed a highly significant increase for all tone-in-noise thresholds for the 24 participants taking part in both the lab and remote experiments. Largest differences were found in the aforementioned most diagnostic stimuli: tone-in-notched noise as well as dichotic tone-in-noise detection. On a population level, results were similar for personally recruited compared to online-recruited participants.

Conclusions: Tone-in-notched-noise and N0S π thresholds turned out to be promising candidates for a quick screening of hearing-in-noise abilities in clinical practice. Significantly worse remote tone-in-noise thresholds indicate the need for additional care when designing online hearing tests, especially for the most cue-specific stimuli. Nonetheless, similar remote testing results for known and unknown test participants indicate the usefulness of online recruitment for epidemiological studies of hearing problems. These results showcase the possibilities of remote hearing testing using tone-in-noise thresholds for a qualitative diagnostic of suprathreshold hearing in an efficient and cost-effective way.

SU196. Exploring the Link Between Frequency Selectivity and Temporal-Coherence–Based Speech Segregation in Sensorineural Hearing Loss

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Background: Individuals with hearing loss commonly report trouble hearing in noise despite using prescriptive amplification. Listening in noise depends on our brains' ability to parse an auditory scene and segregate different sound sources. A key mechanism thought to support segregation is grouping of across-frequency acoustic elements that are temporally coherent (in-phase or comodulated) to form unified perceptual streams or objects. Prior computational modeling work suggests that reduced frequency selectivity, as is common in sensorineural hearing loss (SNHL), can degrade temporal-coherence-based segregation. However, whether broader tuning in SNHL covaries with temporal-coherence-based masking release for speech from background noise has not been directly tested.

Methods: We collected the following measures from native speakers of North American English aged 18-65 years across the continuum of normal-hearing and symmetric SNHL: (i) psychophysical tuning curves (at 4 kHz), (ii) comodulation masking release (CMR) measured for 4 kHz tones in narrow bands of noise, and (iii) speech-in-noise scores while manipulating the temporal coherence between frequency bands containing speech and noise. To date, 5 normal-hearing participants and 6 SNHL participants have completed this study. Importantly, all psychophysical measures used stimuli that were amplified to restore normal loudness perception using a linear hearing aid (Moore and Glasberg, 1998).

Results: Preliminary data suggest that CMR for tonal targets is smaller in listeners with SNHL compared to those with normal hearing. Furthermore, individuals with SNHL also exhibit broader psychophysical tuning-curve tips and reduced tip-to-tail ratios. With data collection and analysis ongoing, individual tuning-curve metrics will be compared to individual temporal-coherence-based masking release data for both speech-based and tone-based stimuli.

Conclusions: We seek to collect data from N=30 participants to test the hypothesis that reduced frequency selectivity covaries with temporal-coherence-based masking release even when audibility is restored.

SU197. Neural Changes After Insc Transplantation Into the Scala Tympani in an Auditory Neuropathy Model

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Background: Studies investigating cochlear hair cell regeneration have been conducted worldwide. However, only a few have been successful, and the clinical application of these therapeutic approaches remains uncertain. Recent advances in cochlear implant techniques are impressive, but maintaining cochlear neural health to improve cochlear implant outcomes remains challenging due to secondary degeneration. Recent studies have demonstrated the direct conversion of mouse somatic cells into induced neural stem cells (iNSCs), which may provide an unlimited source of neuronal cells for autologous transplantation in patients with neurological disorders.

In this study, we evaluated the effects of iNSC transplantation on auditory neural connections in an animal model of auditory neuropathy induced by ototoxic drugs.

Methods: Mongolian gerbils aged 8–9 weeks and GFP-tagged human iNSCs kindly donated by Prof. Shim³ were used. For auditory nerve denervation, 1 mM ouabain solution soaked in gelfoam was placed on the round window (RW) for 30 minutes. Two weeks after ouabain application, human iNSC-GFPs at a density of 6×10^4 cells per 3 μ L were injected into the scala tympani (ST) using a cannula. Auditory function was assessed via auditory brainstem response (ABR) before and after ouabain treatment and surgery. Two weeks after hiNSC transplantation, ABR and electrical ABR (eABR) were recorded, and animals were sacrificed. Hematoxylin and eosin (H and E) staining, epifluorescence analysis of GFP, NF, TuJ, CtBP2, and VGLUT1, as well as quantification of spiral ganglion neurons (SGNs) in the cochlea, were performed.

Results: Ouabain application resulted in hair cells loss and increased ABR thresholds compared to controls. Two weeks after hiNSC transplantation, transplanted iNSCs were located in Rosenthal's canal and increased the neural cell population. ABR thresholds showed no improvement at this time point. The organ of Corti displayed less organized nerve fibers and synaptic structures, with wider intercellular gaps. The average distance between nerve fibers and presynaptic puncta was significantly greater in the hiNSC group. In the central auditory pathway, VGLUT1 expression was significantly increased in both cochlear nuclei, and eABR recordings revealed delayed action potentials in the hiNSC group, indicating partial recovery of afferent neural connections.

Conclusions: Human iNSC transplantation restored neural structures after degeneration, significantly enhancing synaptic connections toward the central auditory pathway, although peripheral synapses were not improved. These findings suggest that combining autologous stem cell transplantation with cochlear implants may improve outcomes and enable cochlear implant use in patients with long-term hearing loss.

SU198. Epigenetic Regulation of EDNRB2, a Key Regulator of Transdifferentiation of Supporting Cells Into Hair Cells, in Explants of Chick Auditory Epithelia

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¹Kyoto University

Background: Our previous studies demonstrated that EDNRB2 regulates fate determination of reprogrammed supporting cells during transdifferentiation of supporting cells into hair cells in collaboration with ATOH1 in chick basilar papilla explants (Takeuchi et al., 2025). This study aimed to elucidate the upstream signals for EDNRB2 expression during hair cell regeneration in chick basilar papilla explants using ATAC sequencing.

Methods: We performed RNA- and -ATAC seq using our explant culture model, in which HCs are entirely lost by exposure to streptomycin (SM) for 48 h. Time course samples for RNA- and ATAC-seq are SM0 (before SM exposure), SM24 (24-h exposure to SM), and SM48 (48-h exposure to SM). For sampling, 25-35 basilar papillae were used for one batch and prepared in duplicate for both analyses. We performed transcription factor (TF)-binding motif scanning for detected enhancer-candidate loci. Based on this, in situ hybridization or immunostaining was done to examine the expression patterns of candidate TFs.

Results: RNA-seq identified 24,888 genes, and ATAC-seq identified 80,366 open chromatin lesions. We identified three candidate loci for EDNRB2 enhancer lesions. TF-binding motif scanning revealed 98 candidate motifs. Filtering by the expression levels of candidate TFs in RNA sequencing data, 23 candidate TFs were identified. Previous studies (Matsunaga et al., 2020; Janesick et al., 2022) have demonstrated upregulation of JAK-STAT signaling pathways in the initial process of hair cell regeneration in chick basilar papillae. Based on this, we chose a couple of TFs, which are associated with JAK-STAT signaling, from 23 candidate TFs for histological evaluation. Phosphorylated STA3 exhibited a similar distribution to EDNRB2 expression.

Conclusions: We conducted an integrated analysis of RNA and ATAC sequencing to explore candidate upstream TFs for EDNRB2. Based on histological findings, functional analyses of candidate TFs will be done in the near future.

SU199. Reducing Sox2 Increases Cochlear Supporting Cell Reprogramming Efficiency

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Background: Sox2 is dynamically expressed in the developing inner ear and expressed in mature supporting cells but not in hair cells. Germline Sox2 haploinsufficiency induces supernumerary hair cells and enhances natural hair cell regeneration in the neonatal cochlea. Mature supporting cells can be reprogrammed to hair cell like cells by combined expression of the transcription factors Atoh1, Gfi1, and Pou4f3 (GAP). To test whether Sox2 influences reprogramming efficiency, we activated GAP and reduced Sox2 in mature supporting cells.

Methods: We bred GAP overexpressing mice to mice carrying either a Sox2 allele flanked by LoxP sites or wild-type Sox2 and crossed these with Pou4f3-DTR mice, to kill hair cells, and Ai3 reporter mice, to track cell lineage. Cre was introduced by intra-labyrinthine AAV-Lfng-CreER or by crossing with Lfng-CreER transgenic mice. Cre recombination with tamoxifen at 3 or 6 weeks induced GAP reprogramming, as well as knocking out of a Sox2 allele. Hair cells

were killed with diphtheria toxin. Mice were euthanized 2 or 9 weeks after hair cell death, and their inner ears harvested for quantitative immunofluorescence microscopy.

Results: Simultaneously reducing Sox2 and overexpressing GAP significantly increased the number of reprogrammed hair cells by 50% at 2 weeks, and by 100% at 9 weeks compared to GAP controls. Reprogrammed hair cells appeared immature and quantifying stereocilia with phalloidin staining showed no difference between genotypes.

Conclusions: Reducing Sox2 in supporting cells at the time of GAP activation significantly increased the number of reprogrammed hair cells. Future therapies for sensorineural hearing loss relying on reprogramming supporting cells could incorporate Sox2 decreasing strategies to increase reprogramming efficiency.

SU200. Exploring Spontaneous Regeneration of Spiral Ganglion Neurons Post-Ablation

Joshua Lin^{*1}, Nhi Nguyen¹, Priya Shah¹, Seiji B. Shibata¹

¹*Keck School of Medicine of USC*

Background: The current consensus suggests that spiral ganglion neurons (SGN) do not spontaneously regenerate in mammals. A recent study has reported spontaneous SGN regeneration following ouabain treatment in adult mice, demonstrating the upregulation of glia markers Sox2/Sox10 within cells expressing the neuronal marker Tuj1. Yet, the precise origin of the spontaneously regenerated SGNs remains unclear. As such, we utilized an inducible Sox2Cre-ER mouse model to enable lineage tracing following SGN ablation with diphtheria toxin (DT).

Methods: Wild-type (C57) and Sox2Cre-ER crossed with Ai9-TdTomato Cre-reporter mice (Sox2Cre-ER+/Td+) obtained from the Jackson Laboratory were used. A single injection of AAV-retro encoding diphtheria toxin receptor gene with GFP (AAV-DTR) was injected via the posterior semicircular canal (PSCC) at P1-2 to express DTR in the SGN. We observed near-total SGN transfection without transfecting the hair cells as early as 7 days post-injection. At 7 or 28 days post-AAV-DTR inoculation, neonatal (P9) and adult (P30) mice were administered DT at 5ng/g and 20ng/g, respectively. Distortion-product otoacoustic emissions (DPOAEs) and auditory brainstem responses (ABRs) were measured before histological assessment and immunohistochemistry of harvested cochleae at 14 days after DT administration. We performed lineage tracing experiments in Sox2Cre-ER+/TdTomato+ mice injected with AAV-DTR at P1-2. At 7 or 28 days post-AAV-DTR inoculation, Tamoxifen was introduced to activate Cre recombinase expression with DT. AAV-DTR-injected, age-matched, saline-administered mice served as controls.

Results: The AAV-DTR-injected wild-type mice, receiving 5ng/g DT at P9 (neonate) and 20ng/g DT at P28 (adult), showed normal DPOAE and increased ABR thresholds at 14 days, consistent with auditory neuropathy. Saline-administered controls demonstrated normal DPOAE and ABR thresholds. Subsequent histology demonstrated a 94% reduction in Tuj+ SGN density (203.86 cells/mm²) in neonates and a 78% reduction in Tuj+ SGN density (894.43 cells/mm²) in adults. Immunohistochemistry indicated co-localization of Tuj and Sox2 signals in 6% of the surviving SGNs in both wild-type deafened neonatal and adult mice, consistent with previous literature. Our preliminary lineage tracing experiment in AAV-DTR-injected Sox2CreER+/Td+

mice demonstrated similar levels of SGN ablation by 14 days after DT administration (92% in neonates and 91% in adults). Consistent with the wild-type immunohistology, we observed TdTomato signals in the few surviving Tuj+ cells in both neonatal and adult mice, indicating spontaneous transdifferentiation from the glial cell lineage to exhibit a neuronal phenotype.

Conclusions: We established a new auditory neuropathy model with temporal control: introduction of 5 and 20ng/g of DT in AAV-DTR-injected mice was sufficient to ablate 78-94% of SGN in neonatal (P9) and adult (P30) mice, sparing the hair cells. Our preliminary fate-mapping experiments in AAV-DTR-injected Sox2Cre-ER+/TdTomato+ mice indicate that spontaneous SGN regeneration from Sox2-positive glial cells occurs in both the neonatal and adult mice following DT-induced SGN ablation. We will further quantify and characterize the regenerated SGN in future experiments.

SU201. Exploring Genes that Enable Hair Cell Regeneration in Zebrafish

Andrea Meza Hernandez*¹

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Background: Hair cells are mechanosensory receptors of the inner ear that are essential for hearing and balance. Damage or loss of these cells in mammals results in permanent hearing loss and vestibular dysfunction due to limited regenerative capacity. In contrast, zebrafish and other non-mammalian vertebrates are able to robustly regenerate hair cells following injury. Understanding the molecular mechanisms that enable hair cell regeneration in these systems may reveal

conserved genetic pathways that could be therapeutically targeted to restore hearing in mammals.

Recent in vitro studies using mammalian inner ear organoid cultures have identified a set of genes implicated in hair cell regeneration through bulk RNA sequencing and single-cell RNA sequencing approaches (Li et al., 2022). These analyses revealed six key regulatory factors: Trim71, Arid3a, Jarid2, Rprm, Nfix, and Zbtb20. These factors are enriched under regenerating conditions compared to controls. They are proposed to play roles in reprogramming supporting cells and promoting hair cell regeneration. While these findings provide important insight, the functional roles of these candidate genes in vivo remain largely unexplored.

This project aims to investigate the in vivo function of zebrafish homologs of selected candidate regeneration genes using the zebrafish larval lateral line system. In addition to possessing hair cells in the inner ear, zebrafish have external accessible hair cells located along their body surface in the lateral line, making them a powerful model for studying hair cell damage and regeneration. Hair cells in the lateral line can be selectively ablated by exposing larval zebrafish to ototoxic compounds such as copper sulfate without damaging surrounding tissues.

Methods: To assess the role of candidate genes in hair cell regeneration, CRISPR/Cas9 mediated mutagenesis will be used to generate zebrafish larvae with targeted gene disruptions. Five-day old CRISPR injected larvae will undergo a standard lateral line regeneration assay in which hair cells are ablated by a one-hour exposure to copper sulfate. Following ablation, larvae will be washed and allowed to recover for two days, a time point at which robust regeneration normally occurs. Regenerated hair cells will then be labeled using the vital dye YO-PRO-1 and quantified to identify defects or enhancements in regeneration relative to wild type controls.

Results: Hair cells count from copper treated CRISPR injected mutants will be compared to untreated CRISPR injected larvae, copper treated wildtype larvae, and untreated wildtype controls. Following phenotypic analysis, genomic DNA will be isolated from individual larvae and analyzed by PCR and sequencing to confirm CRISPR-induced insertions or deletions at the targeted loci.

Conclusions: Together, this work seeks to define the functional roles of conserved regeneration_x0002_- associated genes in vivo and to identify genetic regulators that enable hair cell regeneration.

These findings may advance our understanding of sensory regeneration and inform future strategies for restoring hearing in mammals.

SU202. Processing of Salient Vocalizations in Auditory Cortical Regions Cell Types

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Background: Mice produce a rich repertoire of complex vocalizations across diverse behavioral contexts, such as mating and stress. Playback of these calls elicits distinct behavioral responses and neuromodulator release patterns that vary with the listener's state and the context in which the calls occur. The auditory cortex is thought to relay information about these vocalizations to motor and limbic centers, thereby shaping context-appropriate reactions. However, how such salient vocalizations are represented across cortical subpopulations, and how this representation depends on behavioral state, remain unclear.

Methods: We measured vocalization responses in multiple auditory cortical cell types, including pyramidal excitatory neurons and a subset of inhibitory interneurons expressing Neuron-Derived Neurotrophic Factor (NDNF). These interneurons receive strong neuromodulatory inputs and are thought to modulate cortical activity in a state-dependent manner. Using transgenic mice expressing GCaMP in either pyramidal neurons or NDNF interneurons, we performed widefield and two-photon calcium imaging across auditory cortical regions in awake, head-fixed mice. Mice listened to playback of vocalization syllables recorded during negative (stress), positive

(mating), or neutral (isolation) contexts, while we simultaneously monitored pupil dynamics and locomotion.

Results: Vocalizations evoked robust, widespread responses across primary auditory cortex (A1) and multiple higher-order regions. Two-photon recordings revealed region- and cell type-specific response patterns, with subsets of L2/3 and L4 pyramidal neurons, and NDNF interneurons displaying strong selectivity for specific vocalizations or call classes (e.g., mating versus stress). Notably, many neurons responded robustly to natural vocalizations but not to synthetic sounds with matched spectral content, and small ensembles of vocalization-selective neurons could reliably decode call identity. Ongoing analyses are evaluating how behavioral state modulates the selectivity of these responses.

Conclusions: Together, these findings suggest that auditory cortical circuits encode behaviorally relevant vocalizations in a region- and cell-type-specific manner, and highlight a role for NDNF interneurons in shaping state-dependent processing. By identifying circuit mechanisms for encoding natural communication sounds, this work advances our understanding of auditory perception and lays a foundation for future studies of how internal state and experience shape communication.

SU203. Deep-Neural-Network-Based Hearing Loss Compensation Methods: Personalization and Patient Benefit

Sarah Verhulst¹, Chuan Wen¹, Marjoleen Wouters¹, Morgan Thienpont¹, Nele De Poortere¹, Attila Fráter¹, Guy Torfs¹

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Background: Deep neural network (DNN)-based approaches are increasingly being used to design hearing-loss compensation algorithms. These methods offer low-latency, end-to-end processing suitable for deployment on resource-constrained devices, and they provide greater flexibility in addressing various aspects of sensorineural hearing loss (SNHL) compared to traditional techniques. Their nonlinear structure and ability to optimize via backpropagation make them particularly effective in compensating for deficits caused by cochlear synaptopathy (CS) and outer hair cell (OHC) damage.

Methods: We propose a closed-loop framework for developing augmented-hearing algorithms by minimizing the loss between outputs of differentiable biophysical models simulating normal and impaired auditory-nerve responses. The resulting CoNNear-HA algorithms are personalized using the individual's audiogram and CS profile inferred from auditory physiology. We first benchmark these personalized algorithms against conventional methods using standardized tests, including transfer functions and step/impulse responses. We then evaluate their impact on speech intelligibility in noise while ensuring sound quality remains acceptable. **Results:** We report speech recognition thresholds (SRTs) to 70-dB Flemish Matrix sentences in stationary noise for 20 subjects treated with either CS-specific or combined CS-OHC CoNNear-HA algorithms. While not all participants showed improvement, individual outcomes were analyzed in relation to their audiogram and envelope-following response strength. Post-hoc analysis revealed that the greatest benefit occurred in individuals whose baseline SRTs deviated most from the normal-hearing reference group. To assess performance in a more challenging listening scenario, we tested 40 subjects (20 older normal-hearing and 20 hearing-impaired) on a word recognition task

in babble noise using a signal-to-noise ratio corresponding to their unprocessed SRT. In this condition, most participants benefited from the CoNNear based processing.

Conclusions:

These findings support the integration of DNN-based algorithms alongside conventional methods to enhance hearing in individuals with early to advanced SNHL.

Work supported by FWO G063821N (Machine Hearing 2.0) and EIC transition grant 101058278 (EarDiTech)

Late Breaking Podium

3:00 p.m. - 4:00 p.m.

Meeting Room 209ABC, Floor 2

Moderators: Jeffrey R. Holt and Ronna Hertzano

Molecular Drivers of Directional Regeneration of Spiral Ganglion Neurites and Their Therapeutic Application in Synaptopathy

3:00 p.m. - 3:15 p.m.

Volkan Ergin¹, Wei Wei¹, Chenxi Jin¹, Yi-Zhou Quan¹, Wan Du¹, Stewart Silver¹, Natalie Pelon¹, ZhengYi Chen^{*1}

¹*Massachusetts Eye and Ear, Harvard Medical School*

Background: Spiral ganglion nerve (SGN) degeneration underlies word recognition deficiency and poor cochlear implant (CI) outcomes. While neurotrophic factors, such as BDNF and NT3, promote general neurite growth, their roles in the directional regeneration of spiral neurites and their effect on synaptopathy have been limited. In adult cochlea, while the detachment and retraction of spiral neurites from inner hair cells is generally permanent, we found that adult SGN can regrow their neurites in response to regenerated hair cells (HCs) to form connections. We hypothesized that regenerated HCs secrete molecules to promote this directional regeneration of adult neurites and their identification holds great promise for treating synaptopathy and improving CI performance.

Methods: We used single-cell RNAseq (ScRNAseq) to study transcriptomes in regenerated HCs via reprogramming and ATOH1 overexpression. Bioinformatics was used to identify molecules secreted by regenerated HCs. SGN explant culture assays were used to assess neurite regeneration induced by individual and combined candidates. Dose response and antibody blockade were used to study specificity and potency. In vivo effect on neurite regeneration was studied using noise-exposed and ouabain-treated mouse models with permanent spiral nerve degeneration. AAV-mediated gene therapy was used to deliver the candidates into mice exposed to noise or during aging. ABR thresholds and wave 1 amplitudes were measured with synapses labeled and quantified.

Results: ScRNAseq identified 70 molecules secreted by regenerated HCs, which were reduced to 14 high-probability candidates by bioinformatics analysis. Testing showed a three-molecule combination of Col18a1, Sparc, and Igfbp2 (CSI) to be potent on neurite growth that is dose-dependent and can be specifically blocked by C, S and I antibodies. In adult cochlear explant culture, CSI successfully promoted neurite regeneration and connections with regenerated HCs. Biochemically, C, S and I were shown to interact intracellularly but not as secreted forms. AAV-CSI delivery in adult cochlea, after noise exposure or ouabain treatment, led to potent, directional neurite regeneration toward HCs to form connections. Significantly, AAV-CSI injection into adult inner ear, after noise-induced synaptopathy or with age-related synaptopathy, rescued wave 1 amplitudes and synapses. This rescue effect was mediated by neurite growth and synapse preservation.

Conclusions: Our work, stemming from adult HC regeneration, identified a new class of extracellular molecules COL18A, SPARC and IGFBP2 with potent roles in directional regeneration of damaged neurites in vitro and in vivo. CSI gene therapy achieved therapeutic outcomes in mouse models of NIHL and ARHL. We identified a target-initiated mechanism for neurite regeneration distinct from developmental mechanisms and revealed the regeneration capacity of damaged adult neurites. CSI represents a promising strategy to treat auditory synaptopathy, improve CI outcomes and enhance the treatment of transplanted neuro-stem cells for auditory neuropathy.

Selective Alterations in Intrinsic Excitability of Neurons in the Superior Olivary Complex in a Rat Model of Autism

3:15 p.m. - 3:30 p.m.

Samantha Lauby¹, Aaron Hardman¹, Shaam Anand¹, Nace Golding¹

¹*University of Texas at Austin*

Background: Auditory processing difficulties are often reported from individuals with neurodevelopmental disorders, including autism spectrum disorder. Changes in auditory brainstem functioning, including auditory brainstem responses (ABRs), precede other symptoms of autism spectrum disorder and thus may be a useful biomarker for early detection and intervention. However, the underlying biological mechanisms underlying these changes in ABRs in human infants is largely unknown. Prior research in the autistic brain, especially the cortex, has consistently reported alterations in excitatory/inhibitory balance as a potential biological mechanism underlying the disorder and alterations in auditory brainstem nuclei have also been reported.

Methods: We used a prenatal valproic acid (VPA) exposure model of autism in rats to examine changes in ABRs at postnatal days 35-37. Waveform amplitudes were measured as well as the dB threshold for broadband click and tone stimuli (4-48kHz). Ex vivo patch clamp recordings were made in whole neurons in the superior olivary complex of the auditory brainstem from postnatal days 29-35. Intrinsic membrane properties, including rheobase, input resistance, and time constant were measured in current clamp mode. HCN channel functioning via sag ratio was also assessed in current clamp and their conductance and biophysical properties were assessed in voltage clamp mode. The morphology of the patched neurons was also assessed.

Results: We found that prenatal VPA-exposed offspring have an elevated ABR threshold at all tone stimuli (but not clicks). We also found stimulus-dependent changes in Wave III amplitudes and increased Wave IV/I ratio at all stimuli. At the single-cell level, we found an elevated rheobase, shorter time constant, and higher sag ratio in principal neurons in the medial nucleus of the trapezoid body. This was associated with a higher maximum conductance of HCN channels, suggesting an increase in the total number of HCN channels. We did not find any major alterations in intrinsic membrane properties in principal neurons in the lateral superior olive.

Conclusions: Our results suggest that prenatal VPA-exposed offspring have alterations in central gain and excitatory/inhibitory balance at the superior olivary complex, which can be identified through ABRs. VPA can alter the expression of many genes, so the increase in the maximum conductance of HCN channels in the medial nucleus of the trapezoid body might reflect an underlying mechanism that links prenatal VPA exposure to impaired inhibition in this brain region. Deficits in the detection of speech patterns in challenging listening environments are well-known hallmarks of autism spectrum disorders in humans. The changes we have identified in the sound localization circuitry of the lateral superior olive of rats may provide mechanistic insight into these deficits, and help guide potential strategies for remediation.

A Vestibulothalamic Relay to Posterior Parietal Cortex Defines a Canonical Vestibular Neuraxis

3:30 p.m. - 3:45 p.m.

Todd Mowery*¹, Mark Aziz¹, Justin Yao¹

¹*Rutgers University*

Background: The striatum is the primary input nucleus of the basal ganglia, integrating dense corticostriatal and thalamostriatal projections to regulate action selection and learning. Extensive comparative neuroanatomical work across rodents, nonhuman primates, and carnivores has revealed robust sensorimotor substrates for motor, somatosensory, auditory, and visual processing. In contrast, vestibular sensory pathways remain comparatively unexplored. Our recent work identified a major vestibulothalamic relay from the medial vestibular nucleus (MVN) to the parafascicular nucleus, revealing extensive sensorimotor overlap and a compartmentalized projection zone in the dorsoventral striatum.

Methods: Using retrograde and anterograde AAV tracing in adult Mongolian gerbils, we systematically mapped a second ascending vestibular pathway. Retrograde AAV-GFP (350 nL) was pressure-injected (50 nl/minute) using a Nanoject III at stereotaxic coordinates targeting the center of the lateral dorsal (LD) thalamic nucleus and posterior parietal cortex (PPC). The pipette was left in place for 20 minutes following injection. Anterograde AAV1-GFP (350 nL) was similarly delivered to the center of LD. After three weeks of viral expression, animals were perfused, and brains were extracted, post-fixed, and sectioned coronally at 50 μ m. Sections were float-mounted onto pig-gelatin-coated slides and coverslipped with DAPI-containing mounting medium. All sections were imaged on an Echo Revolve R4 fluorescence microscope using matched exposure and gain settings. Mean fluorescence intensity (arbitrary units) was quantified using the ImageJ histogram tool and exported to Excel. Fluorescence levels were measured

across brainstem, thalamus, cortical, and striatal regions and analyzed in JMP using one-way ANOVA with Tukey's HSD post-hoc comparisons.

Results: We find that projections from MVN target the lateral dorsal (LD) nucleus of the thalamus, which provides a robust thalamocortical input to the posterior parietal cortex (PPC). In turn, PPC corticostriatal projections terminate in a compartmentalized region of the dorsolateral striatum, mirroring the organizational features of the previously described MVN→PF→striatum pathway. A PPC specific compartment just dorsal to auditory and visual inputs to the dorsolateral striatum could represent vestibular striatum.

Conclusions: Together, these results identify a previously unrecognized vestibular circuit linking brainstem, thalamus, parietal cortex, and striatum—defining a canonical vestibular neuraxis in rodents. This pathway provides a new framework for studying vestibular contributions to basal ganglia physiology, cross-modal plasticity, and behavior across species.

Nonclinical Development of a Therapeutic for Usher Syndrome Type 3A Associated Hearing Loss

3:45 p.m. - 4:00 p.m.

Katherine Gribble*¹, Yuan Gao¹, Ye-Hyun Kim¹, Matthew McGill¹, Yukako Asai¹, Lars Becker¹, Vikrant Borse¹, Junaid Syed¹, Janmaris Marin Fermin¹, Izra Hernandez Rivera¹, Michael McKenna¹, Brian Lin¹, Neda Masoudi¹, Bifeng Pan¹, Jean Phillips¹, Kathleen Lennon¹, Danielle Lenz¹, Eva Andres-Mateos¹, Michelle Valero¹

¹*Eli Lilly and Company*

Background: Usher Syndrome Type 3A (USH3A) is an inherited genetic condition affecting hearing and vision. Individuals with USH3A experience progressive bilateral loss of hearing and vision, with clinical diagnosis typically occurring by the age of 10 years. Hearing loss in USH3A is typically symmetric, sensorineural, and progressive, often reaching the level of bilateral profound sensorineural hearing loss (SNHL) by the third or fourth decade of life.

Although USH3A is believed to be caused by a defective clarin-1 protein (individuals with variants in the clarin-1 gene present with USH3A), there are currently no approved pharmacologic therapies for treating either the progressive hearing or vision loss associated with USH3A. The only treatment options for USH3A-associated hearing loss are hearing aids and cochlear implantation, neither of which provide the opportunity to recover lost physiologic hearing nor to halt further progression of physiologic hearing loss. We have developed a therapy that can be delivered to the cochlea to address USH3A hearing loss as discussed below.

Methods: Here we present nonclinical data from mice and non-human primates (NHPs) supporting development of an USH3A therapy for hearing loss. Our USH3A therapy is designed for local delivery to the cochlea. We first characterized the auditory phenotype and disease progression of a mouse model to evaluate its relevance to the USH3A-mediated hearing loss phenotype in humans. Auditory and cochlear function were assessed with auditory brainstem response and distortion-product otoacoustic emission testing, respectively. Cochlear histologic

evaluations were performed to evaluate hair cell survival and sub-cellular hair bundle dysmorphology. We then evaluated the potential for efficacy and otic tolerability of our USH3A therapy across multiple doses when administered at an age when the murine cochlea is mature but before substantial degeneration of hair cells occurs. In NHPs with mature cochleae, we evaluated otic tolerability of our USH3A therapy across multiple doses.

Results: In a mouse model of USH3A-associated hearing loss, we observed a dose-dependent improvement in auditory and cochlear function following administration of our USH3A therapy. Additionally, we observed that increasing doses of our USH3A therapy are well tolerated in the mouse and non-human primate cochlea.

Conclusions: Our results in mice and NHPs support the development of our USH3A therapy as a potential treatment for individuals with USH3A-associated hearing loss.

Symposium 2: New Neurostimulation Strategies for Auditory Restoration

3:00 p.m. - 5:00 p.m.

Ballroom B, Floor 3

New Neurostimulation Strategies for Auditory Restoration

Brice Bathellier, *Institut Pasteur, Institut Audition*

Symposium Description: The cochlear implant (CI) is by far the most successful sensory restoration device, putting the hearing research at the forefront of neurostimulation device development. CI have shortcomings, including their restriction to patients with preserved auditory nerve, and their poor spectral accuracy. Intense research is currently on-going to solve these issues encompassing new algorithms or stimulation methodologies for CI, as well as the conception of brain targeted implants addressing the cochlear nucleus or the even the cortex. The aim of this symposium is to highlight recent progresses made on these fronts, including pre-clinical and clinical research. To account for the diversity of options considered in present research effort we will cover approaches developed at the level of the cochlea, brainstem and auditory cortex. Moreover, we will discuss a large range of research focuses including the impact of AI-based algorithms improving information transfer for CI users, or shaping information at higher level of the auditory system, the development of new electrical stimulators, microfabricated and flexible for higher resolution of stimulation and current progresses in the implementation of optogenetic stimulations techniques for auditory rehabilitation. The symposium will include established specialists and younger researchers demonstrating striking results that prefigure important technological steps with significant improvement of auditory rehabilitation, and will be organized into 6 talks of 15 min and one 30min talk (Moser).

Tania Barkat, *University of Basel*

New and Old Neurostimulation Strategies for Auditory Restoration

Julie Arenberg, *Massachusetts Eye and Ear Infirmary, Eaton Peabody Laboratory*

Individual Abstract: Our research addresses two main challenges that likely limit the speech perception outcomes for individuals with cochlear implants. The first challenge is providing temporal fine structure information. Current implants are primarily encoding the envelope of the acoustic signal, thereby discarding the fine temporal information. There is one strategy that attempts to encode this, but the evidence is mixed about how effective temporal fine structure is transmitted. The second challenge is providing spectral detail by stimulating more localized populations of auditory neurons. Current implants employ the monopolar electrode configuration that induces a broad spread of excitation and more unwanted channel interaction than can be achieved with focused electrical stimulation and potentially with magnetic stimulation through micro-coil arrays. This talk will demonstrate data from animal models demonstrating two approaches to solving these challenges. To provide temporal fine structure, we have applied continuous analog stimulation, which is how cochlear implants were stimulated when they were first developed, but we do this in combination with highly focused electrical fields. Results show that neurons in the inferior colliculus phase lock to the stimulus frequency, and two channels of simultaneous stimulation show small degrees of channel interaction as assessed by quantifying the degree of overlapping neural activation. To provide more spectral detail, we also explore magnetic stimulation from micro-coils of the auditory nerve. Intracochlear magnetic stimulation via a micro-coil produced robust IC activation; the spread of activation was significantly narrower vs. that from monopolar/bipolar electrical stimulation from implanted electrodes. These new stimulation approaches will be discussed within the context of standard cochlear implants in use today.

Bionic Hearing in Mammalian Species Through a Soft Auditory Cortex Implant

Alix Trouillet, *Centre hospitalier universitaire vaudois*

Individual Abstract: By 2050, nearly 700 million people will require hearing rehabilitation according to the World Health Organization. Cochlear implants (CI) are the first line of treatment for severe deafness and they restore meaningful sound perception and speech comprehension to both children and adults. However, significant limitations remain and underscore the urgent need for innovation: 1) CIs do not convey complex hearing experiences such as music or speech in noisy environments, 2) Outcomes are variable across patients with some poor responders with limited improvements, 3) CIs are not indicated for individuals with cochlear and auditory nerve abnormalities as well as central disorders. Direct electrical stimulation of the auditory cortex through an Auditory Cortex Implant (ACI) provides an alternative therapeutic approach tackling some of these challenges. We developed animal models enabling systematic assessment of ACI performance from electrophysiology and behavioral readouts. Applying this framework to rodents, minipigs and non-human primates (NHP), we demonstrated how ACI activates the auditory pathway in a tonotopic fashion, resembling acoustic stimulation. In NHP, the ACI electrode pitch determined the resolution of acoustic percepts enabling a rich encoding capacity of the device. Finally, we measured the behavioral responses to ACI and quantified the auditory nature of the percepts with its acuity and dynamic range. These results reveal the potential of auditory cortical stimulation to restore high-resolution hearing in patients with severe deafness.

Leveraging AI to Improve Speech Information Transmission With Cochlear Implants

Tobias Goehring, *University of Zurich*

Individual Abstract: Cochlear implants (CIs) help many people with severe-to-profound hearing loss hear better, but perceiving speech in a noisy or reverberant environment remains a major challenge for them. I will discuss the limitations in CI hearing responsible for these perceptual difficulties and then describe several avenues to leverage artificial intelligence (AI) to improve speech transmission and perceptual outcomes with CIs. AI methods for the CI frontend processor have shown unmatched potential for enhancing the intelligibility of speech in noisy situations. Our results from several studies using deep learning to improve speech-in-noise perception, include AI systems that can generalise to realistic acoustic situations, benefit from spatial information and let the user choose a specific speaker of interest at runtime. These deep-learning systems achieved meaningful and statistically significant improvements in speech intelligibility even in difficult situations with unseen noise and reverberation or with up to 3 concurrent speakers in background noise. I will present further work on AI-based approaches to tailor these systems specifically for CI speech processing and perception. A hybrid combination between computational models of the auditory system under electrical stimulation and their application as predictors and loss functions for the training of AI-based speech enhancement models is presented. Training such domain-aware speech enhancement models has the potential to further improve performance. Finally, the recent success of powerful speech foundation models in tasks such as automatic speech recognition and translation opens new venues to utilize large-scale speech models to directly assess and compare CI speech processing strategies. First results indicate the potential and feasibility of this approach by applying large speech models to probe speech information transmission in the context of CI simulation and speech processing strategies.

Toward Optogenetic Hearing Restoration

Tobias Moser, *University Medical Center Goettingen*

Individual Abstract: When hearing fails, cochlear implants (CIs) provide open speech perception to most of the currently more than a million CI users. CIs bypass the defective sensory organ and stimulate the auditory nerve electrically. The major bottleneck of current CIs is the poor coding of spectral information, which results from wide current spread from each electrode contact. As light can be more conveniently confined, optical stimulation of the auditory nerve presents a promising perspective for a fundamental advance of CIs. Developing optogenetic stimulation for auditory research and future hearing restoration requires concerted efforts toward development and characterization of appropriate channelrhodopsins, their viral gene transfer to cochlear neurons, as well as medical device engineering. The presentation will summarize the current state of the art and plans for developing gene therapy of the auditory nerve and optical CI as well as their combination for improved hearing restoration by optogenetic sound coding.

Cortical Implants for Hearing Restoration

Tania Barkat, *University of Basel*

Individual Abstract: Hearing loss affects half a billion people worldwide, significantly impairing communication and quality of life. While hearing aids provide adequate support for mild cases, severe hearing loss often requires cochlear implants. Despite their success, cochlear implants suffer from poor spectral accuracy, low acoustic resolution, and ineffectiveness in patients with auditory nerve damage. This study investigates whether cortical implants offer a more effective alternative for auditory restoration.

We implanted surface electrode arrays with 32 contacts (175 μm spacing) onto the auditory cortex of mice and compared them with cochlear implants featuring 4 electrodes (500 μm spacing). To ensure a direct comparison, we analyzed responses from four cortical electrodes, four cochlear implant electrodes, and four frequency-matched acoustic stimuli in normal-hearing mice. Mice were tested on single- and multi-frequency categorization tasks, amplitude modulation categorization, and amplitude modulation in noise categorization. Across all tasks, cortical implants led to superior auditory performance compared to cochlear implants, although learning took longer and followed distinct learning curves. Notably, mice trained with natural sounds could perform the tasks with cortical stimulation without additional training and vice versa, suggesting that cortical stimulation evokes percepts that closely resemble natural hearing.

To investigate the neural basis of these differences, we recorded in vivo electrophysiological responses in the auditory cortex to cortical stimulation, cochlear stimulation, and natural sounds. Neuronal responses scaled with stimulus strength across all conditions. Dimensionality reduction analyses (PCA, t-SNE) revealed that cortical stimulation responses resembled natural sound-evoked responses more closely than cochlear stimulation responses. Linear decoder analyses trained on natural sounds and tested on cortical and cochlear stimulation showed higher classification accuracy for cortical stimulation. Additionally, three-way decoder analyses confirmed that cortical stimulation responses were more similar to natural sounds than to cochlear stimulation, aligning with behavioral findings.

These results suggest that cortical stimulation not only provides better auditory performance but also more faithfully mimics natural auditory processing. The ability of mice to generalize between natural sound and cortical stimulation further supports this claim. While cortical implants show promise as an alternative for hearing restoration, future studies should further investigate learning dynamics, perceptual fidelity, and translational feasibility for clinical application.

A Generic Deep Learning Architecture for Bio-Inspired Encoding of an Auditory Cortical Implant

Brice Bathellier, *Institut Pasteur, Institut Audition*

Individual Abstract: Cochlear implants (CIs) are the most successful sensory neuroprosthesis and help almost a million patients to recover functional hearing. However, their relatively poor spectral and temporal accuracy limits speech intelligibility in noise and music perception. Moreover, CIs do not address pathologies involving auditory nerve loss or cochlear malformation. Because auditory cortex stimulation leads to sound perception, a possible approach to circumvent these issues could be to implant the neuroprosthesis at this level. However, cortical implants would require high-level encoding models to transfer information in a format similar to sound representations in the auditory cortex. To address this challenge, we have developed an AI-based encoder allowing us to recreate and map key features of the

biological auditory code observed in the cortex while ensuring a high information throughput, which is closely measured by reconstructing the waveforms of the encoded sounds. Our approach uses an autoencoder whose latent space matches the number of electrodes on the stimulator and the temporal resolution of auditory cortex activity. The autoencoder is combined with a classifier of human-labelled sound categories that enables the generation of structured pre-semantic representations. The encoder and decoder are based on modern 1D convolution layers explicitly developed for sound processing, yielding efficient training and excellent performance. We demonstrate that this novel hybrid architecture trained on millions of natural sounds can perform sound encoding on hundreds of channels with almost no information loss while generating stimulation patterns matching multiple neural code properties of the auditory cortex. Preservation of information is established by the high perceptual similarity of reconstructed sounds compared to original sounds, for speech, music and natural sounds. The similarity with natural auditory cortex representations is established by using representational similarity analysis on cortical data and on the representation of our encoder. In addition, specific constraints in the training process allow us to reproduce tonotopy. In addition, we demonstrate that single channel activities for well-known sounds have temporal profiles similar to cortical neurons with a variety of onset, offset and sustained responses, as well as tuning properties observed commonly in vivo. Finally, we show that mice can perceive and use the representations generated by our models with good precision. Hence, our approach allows for precisely delineating the design of a potentially successful auditory cortical implant for auditory restoration.

Distinct Cortical Neuronal Populations Are Tuned to Electrical and Acoustic Stimuli in a Chronic Soft Abi Mouse Model

Daniel Lee, *Massachusetts Eye and Ear Infirmary and Harvard Medical School*

Individual Abstract: The auditory brainstem implant (ABI) was developed to restore sound perception in individuals who are not candidates for cochlear implants (CI) due to retrocochlear hearing loss, such as those with Neurofibromatosis type 2 (NF2). Despite its initial promise, ABI performance remains limited—most users continue to experience significant difficulty understanding speech. One potential issue is that neurons in the auditory cortex do not respond to ABI stimulation in the same way as they respond to auditory stimulation. To study this issue, we used a novel chronic ABI model in mouse, allowing us to directly compare neural activation in the contralateral auditory cortex (ACtx) in response to sound and to electrical ABI stimulation.

The model used a flexible three-electrode ABI array to electrically stimulate the dorsal subdivision of the cochlear nucleus (DCN) in awake mice. The strain of mice used in this study express a genetically-encoded calcium indicator in cortical excitatory pyramidal neurons (Thy1-GCaMP6s or VGlut1-GCaMP8s). Two-photon imaging was performed through a cranial window over ACtx to measure sound and ABI electrically-evoked responses at both the single-neuron and populational levels. Neurons studied were within L2/3 (~250µm depth) of ACtx. Responses to amplitude modulated (AM) sounds and ABI stimulation were compared across a range of modulation depths.

ABI stimuli elicited diverse calcium responses in ACtx, including neurons with rapid transient activation, delayed sustained responses and stimulus-evoked suppression – response profiles

broadly similar to those evoked by sound. Approximately 30% of neurons responded to ABI and a similar proportion to sound stimuli, however only a small fraction responded to both, suggesting largely non-overlapping populations. Although many neurons displayed sharp tuning to specific modulations in acoustic and electrical stimuli, tuning preferences for AM-ABI and AM-sound were often mismatched at the single-neuron level. These findings suggest that although the ACtx is capable of decoding ABI inputs, these signals may not be immediately aligned with natural auditory coding strategies. This mismatch may contribute to the limited perceptual performance of ABI users and highlights the potential for cortical plasticity to enhance outcomes—aligning with clinical reports that ABI performance improves with continued device use. Ongoing work aims to determine whether behavioral training can reshape cortical tuning to ABI stimuli and enhance perceptual performance.

Podium 7: Neural Correlates of Tinnitus

3:00 p.m. - 5:00 p.m.

Meeting Room 208ABC, Floor 2

Moderators: Joel Berger and Nour Alsabbagh

Integrating Afferent and Efferent Auditory Assessments to Explore Central Gain and Hidden Hearing Loss in Tinnitus

3:00 p.m. - 3:15 p.m.

Pauline Devolder^{*1}, Hannah Keppler², Sarah Verhulst¹

¹*Hearing Technology at WAVES, Ghent University*, ²*Ghent University, Ghent, Belgium*

Background: Tinnitus is a symptom that is often associated with aging or excessive noise exposure. Emerging evidence suggests that interactions between peripheral hearing loss and central gain changes can alter central auditory and non-auditory regions, potentially contributing to tinnitus. Peripheral and central imbalances may affect both upward neural encoding and downward feedback pathways. This study combines novel afferent and efferent auditory measurements to examine how these factors interact and shape auditory encoding in individuals with tinnitus.

Methods: To assess afferent neural encoding from the cochlea to the inferior colliculus, the protocol included auditory brainstem responses (ABRs), envelope-following responses (EFRs), and frequency-following responses (FFRs) to low-frequency harmonic complex tones and speech syllables. Low-frequency FFRs allow us to differentiate between envelope and temporal fine structure encoding. The efferent reflex pathways were assessed using wideband click-evoked middle-ear muscle reflexes (MEMR) and wideband medial olivocochlear reflexes (MOCR). Additionally, several behavioural and tinnitus-related measurements were implemented. 120 participants were included: sixty with chronic tinnitus (noise-induced or idiopathic) and sixty age-matched controls, subdivided into young (18 – 35 y/o) normal-hearing, older (45 – 65 y/o) normal-hearing, and older hearing-impaired groups. Group comparisons evaluated the effects of tinnitus, age, and hearing loss on auditory processing.

Results: Participants with tinnitus had enhanced neural envelope encoding and improved behavioural low-frequency vowel discrimination. While typical listeners, as reported in previous studies, usually rely on envelope information to encode speech in noise, participants with tinnitus showed a correlation between neural temporal fine structure encoding and low-pass speech-in-noise scores. As expected, older listeners showed reduced responses on almost all measurements, which interacted with tinnitus-related enhancements. MEMR analyses revealed significant age-related effects but no direct tinnitus-related differences. This suggests that efferent function alone is insufficient to explain tinnitus-related enhancements and central gain.

Conclusions: These findings highlight the importance of considering interactions between peripheral damage, aging, and central gain in tinnitus research. By integrating afferent and efferent measures, this study establishes a comprehensive framework for interpreting changes in subcortical auditory encoding related to tinnitus. This framework also paves the way for future research on central gain in relation to aging, tinnitus, and hyperacusis.

Work supported by FWO G068621N AuDiMod.

Neural Plasticity Following Restricted Peripheral Cochlear Deafferentation in Ferrets

3:15 p.m. - 3:30 p.m.

Victoria Bajo*¹, Joshua R. Gold¹, Sara Rolle Sonora¹, David N. Furness², Linus Milinski¹, Andrew J King¹, Fernando R. Nodal¹

¹*University of Oxford*, ²*Keele University*

Background: Subjective tinnitus is the perception of a sound in the absence of an environmental source, and is thought to derive from maladaptive plasticity following hearing impairment. Our aim was to investigate whether primary auditory cortex has a causal role in a model of trauma-induced tinnitus.

Methods: Our longitudinal experimental design comprised gap-in-noise behavior using positive operant conditioning and auditory brainstem response (ABR) recordings to detect the presence of tinnitus before and after making a restricted unilateral mechanical lesion in the cochlea. Normal hearing ferrets underwent behavioral testing on a two-alternative forced choice paradigm and ABR measurement prior to spiral ganglion lesion. Post-lesion, the animals were re-tested behaviorally and ABRs were recorded at two time points (1 week and 3 months). Animals were tested again in the gap-in-noise task after deficient replicant AAV virus containing ArchT tagged with GFP was injected bilaterally in the primary auditory cortex (A1) and 200 μ m optical fibres were implanted at the surface of A1 to silence cortical activity. Pulses of green light ($\lambda = 532$ nm, 8-10 mW) from a compact diode-pumped solid-state (DPSS) laser were randomly paired with the sound stimuli and applied to A1 on the right, the left or both sides of the brain in 50% of the trials presented. To investigate neuroplasticity in A1 neurons, recordings were performed using Neuronexus probes in the two hemispheres and compared with recordings from naïve animals.

Results: Spiral ganglion lesion resulted in a heterogeneous change in gap-in-noise detection behavior across animals, with some ferrets displaying temporal processing impairments and

others showing post-lesion adaptation. These behavioral outcomes correlated with particular ABR features, indicating a possible predisposition towards adaptation/maladaptation. Interestingly, optogenetic silencing of A1 contralateral to the lesioned cochlea showed a rescue effect on the behavioral markers of impairment.

Spiral ganglion lesions appear to reduce the afferent drive to the A1 with recorded units showing lower spontaneous firing rates than in naïve controls. Importantly, when behavioral status was considered, the spontaneous firing rate of A1 neurons from cases that did not show a behavioral impairment was significantly lower and more synchronized than that in animals that showed a behavioural impairment. Frequency remapping in A1 was not observed following spiral ganglion lesions, though in control cases, the right hemisphere showed a greater representation of low frequencies than the left hemisphere, whereas this inter-hemispheric asymmetry was not observed in the lesioned cases, where A1 units were significantly more narrowly tuned than those in control animals.

Conclusions: These results suggest that A1 shows evidence for behaviorally-relevant deafferentation-related neuroplasticity, suggesting possible targeted strategies for remediation of the maladaptive processing potentially underpinning tinnitus in humans.

Development of a New Tinnitus Model Using Designer Receptors Exclusively Activated by Designer Drugs (DREADD)-Based Chemogenetic Technology

3:30 p.m. - 3:45 p.m.

Joseph Pinkl^{*1}, Giovana Da Silva², Harry Wang², Jianxin Bao¹

¹Gateway Biotechnology Inc., ²Duke University

Background: Tinnitus research at the preclinical stages is difficult to manage due to a lack of understanding of its neurological mechanisms and limited reliable tests to identify tinnitus in animal models. Evidence obtained from both animal and human studies suggest that tinnitus results from the dysfunction of several neuronal networks. Here, we aimed to develop a new pathological model of tinnitus by directly modulating neuronal activity of the inferior colliculus (IC) in mice using Designer Receptors Exclusively Activated by Designer Drugs (DREADD)-based chemogenetic tools, a class of engineered proteins that are activated by synthetic ligands. Validation measures were completed using a behavioral-based tinnitus detection method called sound-based avoidance detection (SBAD). Additionally, we supplement SBAD data with deep learning video tracking analyses to profile tinnitus-associated passive behavior.

Methods: Eight adult C57BL/6J mice received bilateral stereotaxic micro-injections of AAV8-CaMKIIa-hM3D(Gq)-mCherry (AAV) at 50-150 nL doses in the central nucleus of the IC (AP: -5.00 mm, ML: +/- 1.00 mm, DV: -1.50 mm). After one month of recovery, animals underwent 15 days of SBAD training followed by 6 consecutive days of SBAD testing. On every other day of testing, animals received intraperitoneal (i.p.) injections of clozapine-N-oxide dihydrochloride (CNO), a chemogenetic actuator, at a dose of 5 mg/kg, one hour before testing. On all other days of testing, animals received .5ml injections of .9% saline as a control. Video tracking was completed using an overhead camera capturing animal movement within a 15inx8inx5in cage. Video recordings were obtained from each animal individually under two conditions – 1 hour post i.p. CNO injections and 1 hour post i.p. saline injections.

Results: For AAV injected animals, there was a significant main effect of injectant type (saline vs CNO) for the SBAD silent trials only. However, there was no significant main effect of AAV dose and no significant interaction between injectant type and AAV dose for both silent and sound trials. Silent trial scores post-CNO injection ranged from approximately 50-100% with seven of the eight IC AAV injected animals yielding scores that indicated tinnitus-associated behavior. Video tracking analyses indicated 9 distinct metrics of animal behavior that yielded significant differences between injectant type (saline vs CNO).

Conclusions: SBAD scores suggest that selective modulation of IC excitatory neurons via chemically activated DREADDs can lead to tinnitus-associated behavior. Video tracking analyses identified 9 behavioral metrics that may be associated with tinnitus percept. Future studies will continue to build these tinnitus-associated behavioral profiles. Because IC hyperactivity is implicated in many tinnitus studies, excitatory DREADD applications in auditory pathways could be reliable experimental tools for tinnitus research.

Identify Tinnitus-Related Neural Activity With a Machine Learning Classification Model

3:45 p.m. - 4:00 p.m.

Jinsheng Zhang^{*1}, Na Zhu², Hao Luo³

¹Wayne State University, ²University of Michigan, ³Henry Ford Health

Background: Tinnitus is a prevalent medical condition that affects many millions of people. Efforts have been made to better understand its underlying mechanisms and develop treatment. However, there is a lack of reliable biomarker for diagnosing tinnitus. In animal studies, it has been shown that behavioral evidence of tinnitus is associated with hyperactivity often in the forms of higher firing rate. However, there is a significant difference among individuals. That is, firing rates alone may not be the only factor in determining the presence or absence of tinnitus. In this study, we adopted an integrated approach, leveraging machine learning (ML) to examine spike rates and spike patterns, in order to separate animals with and without tinnitus. Ultimately, our goal is to develop a ML tool that can reliably identify biomarkers for tinnitus by evaluating waveform measurements from neural activity.

Methods: In this study, spontaneous activity was recorded in the auditory cortex (AC) of seven rats with known tinnitus status using gap prepulse inhibition of the acoustic startle paradigm: four displaying behavioral evidence of tinnitus, and three without tinnitus. During the signal processing, local field potentials were analyzed to determine the mean and standard deviation, providing data's values and range. Statistical measures were used to detect spikes in the time domain. A short segment of the waveform was extracted at each spike location, ensuring the segment capturing the entire spike waveforms. A total of 10,000 spike samples were used to train and test a supervised ML model.

For the ML process, a model was developed using four input features: spike firing rate, spike amplitude, prominence, and width. The output prediction was a binary value: "0" indicating no tinnitus, and "1" indicating tinnitus was observed. Seventy-five percent of the data were used for model training, while the remaining 25% was used for validation. The ML algorithms included

decision trees and support vector machines (SVM), and their validation accuracy was compared. The importance of the four features in model building was assessed using various ranking algorithms, such as Minimum Redundancy Maximum Relevance (mRMR) and the Chi-squared test.

Results: Our results demonstrated that the ML can identify the presence of tinnitus with an accuracy of approximately 90% in several methods, including the SVM, k-nearest neighbors (KNN), and tree-based models. Among these, the bagged tree algorithm demonstrated the highest accuracy. Additionally, the firing rate, spike amplitude, and prominence are the three most important input features.

Conclusions: The machine learning model may be further developed to an effective tool for identifying a biomarker of tinnitus.

Neuroinflammation of Central Auditory and Limbic System Induced by Blast Exposure

4:00 p.m. - 4:15 p.m.

Wei Sun^{*1}, Li Li¹, Guangdi Chen¹

¹*University at Buffalo*

Background: Tinnitus and memory deficits are commonly seen in veterans who have traumatic brain injury (TBI) during their service. There is supporting evidence suggesting that blast-caused neuroinflammation may underline these symptoms.

Methods: Two-choice paradigms of the operant conditioning tests were used to test tinnitus and hyperacusis in adult Sprague Dawley rats induced by blast exposure. Immunocytochemistry was used to detect brain inflammation signals in the central auditory system and hippocampus using microglial cell markers. To isolate blast induced brain injury affected by peripheral auditory damage, the ear canals of the rats were plugged during the blast exposure. Rats without blast exposure were used as controls.

Results: Rats in the TBI group with ear protection did not develop hearing loss. The reaction time showed a significant decrease, indicating increased sound sensitivity, in blast exposed rats with ear protection. In the TBI groups, increased neural inflammation expression of IBA-1 and CD-68 signals were detected in both the hippocampus and auditory pathways, compared to controls.

Conclusions: Our data found blast-induced neuroinflammation in the central auditory system and hippocampus area may be linked to tinnitus and memory deficits after blast exposure.

Sound-Evoked Pain Behavior Analyzed by Machine Learning Exceeds a Threshold Set by a Known Pain Model

4:15 p.m. - 4:30 p.m.

Benjamin Seicol^{*1}, Amelie Valles², Bryan Djunaidy², Elisabeth Glowatzki¹, Megan Wood³

¹*Johns Hopkins School of Medicine*, ²*Johns Hopkins University*, ³*Vanderbilt University School of Medicine*

Background: The auditory pain threshold in humans is commonly reported to be ~120 dB (Sherlock and Formby, 2005). This threshold was thought to be similar in mice, and loud sound exposure has been routinely employed for noise-induced hearing loss in rodent models. To investigate whether high-intensity sound evokes behaviors that indicate pain, calcitonin gene-related peptide (CGRP) was used to induce migraine-like pain as a comparative tool to validate our sound-evoked behavior assay. CGRP was selected as the painful stimulus of interest due to its known expression in the auditory system (Cabanillas and Luebke, 2002; Schrott-Fischer et al., 2005), its ability to lower auditory thresholds (Rahman et al., 2025; Le Prell et al., 2021), and its association with phonophobia (heightened auditory sensitivity or pain), a symptom frequently reported by migraine sufferers (Lipton et al., 2024).

Methods: C57BL/6J mice aged 6 – 10 weeks old were recorded inside a custom-built clear container in a sound chamber. CGRP protocol: Mice were first recorded for 10 minutes to establish baseline behavior. Behavioral recordings were conducted following CGRP injection (100 µg/kg, i.p.) or saline during the middle of the migraine period (20–40 minutes post-injection), which included the expected peak at approximately 30 minutes post-injection (Rea et al., 2018). Sound protocol: Mice were recorded for 10 minutes to establish a baseline and then exposed to 2 – 20 kHz sound for 2 minutes ranging from 70 – 120 dB SPL interleaved with 2 minutes of no sound. Deep learning was applied to automatically place thirteen pose-estimated points and connecting skeletons on the face and body of the mice. Six facial grimace measurements (La Moëne and Larson, 2023) and four new body positions parameters were separately added together to calculate two cumulative scores.

Results: After CGRP injection, both Facial Grimace and Body Position Scores were statistically distinct from baseline recordings, indicating pain. Saline-injected controls did not show a significant change in Facial Grimace or Body Position Scores. Pain thresholds for sound were similarly defined by a significant difference from baseline in both additive Facial Grimace and Body Position Scores. Normal hearing mice were found to have auditory pain thresholds of 100 dB SPL based on these criteria that also exceeded the magnitude of change in CGRP-injected mice. Animals returned to baseline behavioral levels during periods of no sound following sound presentations, except for post 120 dB SPL-exposure.

Conclusions: This study highlights the need for caution when exposing animals to sound levels above 100 dB without appropriate anesthesia or analgesia to mitigate potential pain. Machine learning analysis of video recorded mice during sound presentation proved to be an effective approach to measuring auditory pain in mice and could be applied to hyperacusis models.

Use of Opm-Meg for Identification of the Neural Correlate of Tinnitus

4:30 p.m. - 4:45 p.m.

Marlies Knipper^{*1}, Rodrigo Donoso-San Martín², Stephan M. Wolpert³, Stefan Fink³, Paul Delano², Christoph Braun⁴, Lukas Rüttiger³

¹*University Hospital Tuebingen*, ²*Laboratorio Neurobiología de la Audición, Facultad de Medicina, Universidad de Chile*, ³*Tübingen Hearing Research Centre, University of Tübingen*,

⁴*MEG-Center, University of Tübingen, Otfried-Müller-Straße. and Hertie Institute for Clinical Brain Research*

Background: Acquired auditory processing disorders including age dependent hearing loss, speech discrimination deficits, tinnitus or hyperacusis, require a personalized diagnosis to assign the individual cause within the auditory hierarchy to either the periphery, subcortical, or distinct cortical or cortico-fugal neuronal dysfunctions. The good functioning of the feedforward and feedback PV-IN network is an essential precondition for audition that above all senses relies on high-speed information flow (Zajac IT and Nettelbeck T, 2018). Therefore, we hypothesize disease-specific deficits in temporal intracortical network function in auditory circuits.

Methods: We studied fast auditory processing in tinnitus subjects with or without the comorbidity of hyperacusis using auditory stimuli evoking time-sensitive cortical responses. These responses were recorded using a time-sensitive OPM-MEG.

Results: We expect this method to become an efficient diagnostic strategy to fathom peripheral or central contribution of the distinct auditory impairments in the future to improve individualized targeted interventional therapies.

Conclusions: Here we will present preliminary results demonstrating the usability and function of the OPM-MEG for hearing research in a clinical setting.

Acknowledgment and funding: This work was supported by ERA-NET NEURON JTC 2020: BMBF 01EW2102 CoSySpeech and the Tübingen Research Take off Program (TRT) of the Brasilien-Lateinamerika Zentrum, University of Tübingen.

Permanent Reversal of Noise-Induced Tinnitus by 28-Days of Ebselen Treatment is Related to Significant Reductions in Outer Hair Cell Loss

4:45 p.m. - 5:00 p.m.

Kushal Sharma¹, Annie Jia¹, Rende Gu¹, Sao-Mai Gahr¹, Ryan Longenecker¹, Jonathan Kil*¹

¹*Sound Pharmaceuticals, Inc.*

Background: Noise-induced hearing loss (NIHL) is a major risk factor for developing noise-induced tinnitus (NIT). Prior human studies have shown that 4-days of ebselen can prevent acute NIHL in young adults and 21-28 days of ebselen can improve hearing and tinnitus in aged adults with Meniere's disease. We previously showed in young and old mice that 4 or 14-days of ebselen treatment can temporarily reduce or partially reverse NIT. The goal of this current study was to determine if 28-days of ebselen treatment can permanently reverse NIT in young (3 to 4-month) and old mice (12 to 15-month) and if it is related to outer hair cell (OHC) loss.

Methods: Two ages of CBA/CaJ mice (N=60) were utilized: 3 to 4-month-old (N=30) and 12 to 15-month-old (N=30). Each age group was further divided: Group 1: 3-6 month (N=6) and 12-15 month (N=6) served as an unexposed controls with no ear occlusion; Group 2: 3-4 month (N=24) and 12-15 months (N=24) received a temporary unilateral occlusion of an external ear canal before a brief narrowband noise exposure (awake, 112 dB, two hours, octave-band noise centered at 8-kHz). Baseline ABRs and Gap-Induced-Prepulse-Inhibition of the Acoustic Startle Reflex (GPIAS) were assessed prior to and three months after noise to determine changes in

GPIAS ratios (behavioral marker for NIT). The mice that developed NIT were treated with 28-days of ebselen (10/mg/kg/d/ip) and reassessed at 1, 10, and 30-days post-treatment. Cochlear histopathology was performed using Myosin-7a immunolabeling to identify OHC loss.

Results: Three months after noise exposure, permanent threshold shifts were observed in the open ears of all noise-exposed mice across three tested frequencies (16-32 kHz). GPIAS showed frequency specific changes (NIT) in a subset (N=13) of the noise-exposed mice (N=48). After 28-days of ebselen, gap detection deficits were significantly reduced to pre-exposure levels in 12 of the 13 (92%) NIT mice at 1-day, in 10 of 12 (83%) NIT mice at 10-days, and in 9 of 11 (82%) NIT mice at 30-days post-treatment. Mice with permanent reversal of NIT showed significantly less OHC loss (3 complete rows) than those with temporary reversal: 0.073 ± 0.07 mm vs 0.660 ± 0.06 mm, respectively ($p=0.0013$).

Conclusions: 28-days of ebselen treatment can permanently reverse gap detection deficits to baseline or pre-noise exposure in 82% of mice that developed NIT, 3-months after noise exposure and the development of NIHL. In addition, the significant reduction in complete OHC loss suggests a broad therapeutic window by which to prevent injured OHCs from dying. This work is the first demonstration of an anti-inflammatory drug permanently reversing NIT after the development of age-related hearing loss. These data further support ebselen as a treatment for NIHL and NIT in young and old adults.

gEAR Session: Advanced Workshop

4:15 p.m. - 5:15 p.m.

Meeting Room 209ABC, Floor 2

Join us for an exciting workshop where you'll learn directly from leading investigators how to harness the power of the **gEAR platform** to explore cutting-edge, newly generated **multi-omic datasets** in our field—and accelerate your own research. We'll kick off with a brief introduction from the gEAR team, followed by three, brief, expert-led sessions:

- **Brian Herb** - Showcasing the **Hearing Restoration Project's new collection** of multi-omic datasets now available in gEAR. Brian will demonstrate how to use the **gEAR projection tool** for transfer learning and for identifying shared molecular features across diverse datasets.
- **Matthew Kelley** - Presenting the **first release of CoMAP**, the NIDCD's cochlear multi-omic and spatial transcriptomic atlas. Matthew will guide you through co-exploring **transcriptomic and spatial transcriptomic data** using gEAR's integrated tools.
- **Celia Bloom** - Sharing a **new gene regulatory network** from the lab of Litao Tao and demonstrating how to use the **new epigenomic viewer** in gEAR. Celia will walk through how to visualize and connect **DNA accessibility, epigenetic marks, CUT&RUN, and Hi-C data** with transcriptomic datasets—all within the platform.

Advanced gEAR Workshop

Brian Herb, *University of Maryland School of Medicine*

Presenting the First Release of CoMAP, the Nidcd's Cochlear Multi-Omic and Spatial Transcriptomic Atlas. Matthew Will Guide You through Co-Exploring Transcriptomic and Spatial Transcriptomic Data Using Gear's Integrated Tools

Matthew Kelley, *NIH/NIDCD*

Sharing a New Gene Regulatory Network from the Lab of Litao Tao and Demonstrating How to Use the New Epigenomic Viewer in Gear. Celia Will Walk Through How to Visualize and Connect Dna accessibility, Epigenetic marks, CUT&RUN, and Hi-C Data with Transcriptomic datasets—all within the Platform.

Celia Bloom, *Creighton University*

Inner Ear Courses

6:30 p.m. - 7:30 p.m.

Meeting Room 208ABC, Floor 2

Transforming Vestibular Care Through Clinical Training: A Framework from the EU MSCA PROVIDE Doctoral Network and the Bárány Society's Vestibular Medicine Program

Sonja Pyott, *Carl von Ossietzky University of Oldenburg*

Transforming Vestibular Care through Clinical Training: A Framework from the Eu Msca Provide Doctoral Network and the Bárány Society's Vestibular Medicine Program

Raymond van de Berg, *Maastricht University Medical Center+*

JAX Course

Matthew Kelley, *NIH/NIDCD*

Inner Ear Courses: NIDCD EARssentials Course

Elyssa Monzack, *NIDCD*

Biology of the Inner Ear

Katie Kindt, *NIH/NIDCD*

NIDCD Workshop: Changes at the NIH for Established Investigators

6:30 p.m. - 8:00 p.m.

Meeting Room 209C, Floor 2

Over the past year, the NIH has introduced several significant policy changes that directly affect current NIDCD awardees. This workshop is designed to provide clear guidance on these updates and equip awardees with the knowledge and strategies needed to successfully navigate the evolving requirements.

Changes at the NIH for Established Investigators

Meeting Room 209C, Floor 2

Janet Cyr, *NIH/NIDCD*

Samantha Tempchin, *NIH/NIDCD*

Becky Wagenaar-Miller, *NIH/NIDCD*

SEED.NIH.GOV Helping Innovators Turn Discovery Into Health

6:30 p.m. - 8:00 p.m.

Ballroom B, Floor 3

SEED grants are made to U.S.-based companies with fewer than 500 employees and have been the largest source of early-stage capital for life sciences in the U.S., specifically to support research and development within small businesses. The grant program expired on Sept 31, 2025, so the workshop will begin with an update on its current status.

Seed.NIH.GOV Helping Innovators Turn Discovery Into Health

Ballroom B, Floor 3

Roger Miller, *NIDCD/NIH*

Monday, February 9, 2026

Podium 8: Molecular Regulation of Hair Bundle Assembly and Function

8:00 a.m. - 10:00 a.m.

Ballroom B, Floor 3

Moderators: Shefin George and Abigail K. Dragich

Formins and Ankle-Link Complexes Regulate Stereocilia Length in Auditory Hair Cells

8:00 a.m. - 8:15 a.m.

Chun-Yu Tung¹, Xiayi Liao¹, Benjamin Perrin*¹

¹*Indiana University - Indianapolis*

Background: Stereocilia are actin-based protrusions that form a staircase-like bundle on auditory hair cells, essential for mechanotransduction. Their cores consist of long, unbranched actin filaments whose barbed ends reside at the tips. Formins nucleate unbranched actin filaments and elongate them from their barbed ends, so we hypothesized that formins drive stereocilia elongation.

Methods: We treated cochlear explants with the pan-formin inhibitor SMIFH2, then assessed stereocilia morphology by scanning electron microscopy (SEM), examined protein localization by immunofluorescence, and measured actin incorporation using phalloidin labeling. Finally, we performed injectoporation of EGFP-PDZD7 or EGFP-MYO7A constructs to test functional relevance.

Results: If formins elongate the F-actin in the core, then inhibition would result in shorter stereocilia. Instead, SMIFH2 induced stereocilia elongation, suggesting formin inhibition enhanced actin assembly at tips. FMN1 and DAAM1, the most abundant formins per mRNA sequencing, localized at stereocilia bases with ankle-link proteins PDZD7 and MYO7A. SMIFH2 disrupted this localization, redistributing these proteins along stereocilia and to tips. EGFP-PDZD7 overexpression mirrored SMIFH2, causing PDZD7 and MYO7A tip accumulation and elongation. Wild-type EGFP-MYO7A had minimal effects, but a Myo7a mutant with increased activity that is associated with hearing loss did increase stereocilia length.

Conclusions: FMN1 and DAAM1 anchor ankle-link complexes that restrain tip-ward actin polymerization. Disruption of this anchoring, either pharmacologically or by PDZD7 overexpression, released the restraint and increased stereocilia elongation, likely via altered MYO7A activity. This novel regulatory pathway connects ankle-link organization to stereocilia length control.

Tip Link Re-Assembly and Re-Anchoring During Stereocilia Cytoskeleton Remodeling in Inner Ear Hair Cells

8:15 a.m. - 8:30 a.m.

Ana I. Lopez-Porras^{*1}, Sara Gonzalez-Velez¹, Patricia Quiñones², Abigail Dragich¹, Sebastiaan Meenderink³, Luis Rendon-Orozco¹, Dolores Bozovic⁴, Gregory Frolenkov¹, A. Catalina Velez-Ortega¹

¹*University of Kentucky*, ²*University of Southern California*, ³*VA Long Beach Healthcare System*,
⁴*California NanoSystems Institute, UCLA*

Background: Apical surfaces of inner ear hair cells feature stereocilia bundles, formed by highly organized microvilli-like projections arranged in rows of increasing height. The tips of shorter stereocilia are connected to the sides of taller neighbors via tip links. Deflection of these bundles increases tip link tension, activating mechano-electrical transduction (MET) channels. Even at rest, tip links remain tensioned, maintaining optimal mechanosensitivity and allowing a small influx of cations. It has been shown that changes to this resting MET current influence stereocilia cytoskeleton morphology (Velez-Ortega et al., eLife, 2017), and that auditory hair cells can modulate their mechanosensitivity through such cytoskeleton remodeling (Dragich et al., in review, 2025). These findings, however, assume an upper tip link anchoring point with limited mobility. Here, we induced exaggerated stereocilia remodeling (> 150 nm shortening) in the shorter rows of the hair bundle to investigate whether tip links break (and reform) or slide down during this dynamic process, and to further test the mobility capacity of the upper tip link insertion site.

Methods: The organ of Corti from mice, as well as the sacculus and amphibian papilla from bullfrogs, were dissected and incubated with pharmacological blockers of MET channels to initiate stereocilia remodeling. Samples were then processed for scanning electron microscopy (SEM) to visualize/quantify tip links or embedded for focused ion beam (FIB)-SEM to assess upper tip link densities (UTLDs). We also assessed MET currents via whole-cell patch-clamp recordings and mechanical stimulation of hair bundles.

Results: During initial stages of stereocilia remodeling (~150 nm of shortening), there was a significant reduction in tip link counts in all hair cell types evaluated, accompanied by changes in the appearance of surviving tip links and reduced MET currents. Consistent with Grati and Kachar (PNAS, 2011), the UTLD remained at or near its original position, and thus significantly far from the tips of shorter stereocilia. At later time points (up to 500 nm of stereocilia shortening), tip link counts, their appearance, and MET amplitudes were fully restored. In addition, the UTLD was observed significantly lower from its original location, at a position consistent with the new tip link insertion site.

Conclusions: We show that tip links and their associated structures can remodel in response to significant changes in stereocilia morphology. The initial stages of stereocilia shortening disrupt tip links, likely due to limited mobility of the UTLD. After extensive stereocilia shortening, however, new tip links form and UTLDs are found at the new tip link insertion points. These findings indicate that the UTLD can reform/relocate when the tip link reassembles at a new location, revealing a previously unrecognized mechanism of plasticity of the upper tip link insertion point in hair cells.

Supported by University of Kentucky College of Medicine and Department of Physiology.

The Role of MYO7A Isoforms in Tuning Hair Cell Function

8:30 a.m. - 8:45 a.m.

Sihan Li^{*1}, Jinho Park², Andrew Mecca³, Giusy Caprara³, Natchanon Sittipongpittayaemail¹, Gloria Sheynkman¹, Edward Egelman¹, Anthony Peng³, Jonathan Bird², Jung-Bum Shin¹

¹*University of Virginia*, ²*University of Florida*, ³*University of Colorado Anschutz Medical Campus*

Background: In auditory hair cells, tip-link tension is essential for the sensitivity of the mechano-electrical transduction (MET) process. Our previous study provided evidence that the unconventional Myosin VIIa (MYO7A) is essential for tensioning the tip-link MET complex. We further discovered two MYO7A isoforms with unique N-terminal extensions in inner and outer hair cells (IHCs and OHCs), with their expression pattern correlating with reported differences in tip-link tension. The goal of the present study was to explore the hypothesis that the differential expression of functionally distinct MYO7A isoforms directly affects hair cell physiology such as tip-link tension and resting open probability, and hearing sensitivities across hair cells at different frequencies.

Methods: Kennix long-read sequencing by PACBIO was performed on cochlea cDNA to identify new transcript isoforms. MET currents were recorded in response to fluid jet stimulations, and hair bundle motion was monitored by a high-speed camera. SEM and immunofluorescence microscopy were used to investigate hair bundle morphology. ATPase hydrolysis assay and actin gliding assay were conducted to determine the motor activities of MYO7A isoforms. Cryo-EM was applied to resolve the structure of mouse MYO7A isoforms.

Results: Kennix sequencing identified two major isoforms of Myo7a in the auditory system: a widely studied canonical isoform (MYO7A-C) and a previously unreported novel isoform (MYO7A-N). Analysis of isoform-specific HA KI mice revealed that IHCs predominantly express MYO7A-C, and a significantly lower level of MYO7A-N. In OHCs, MYO7A-C and MYO7A-N showed an opposing gradient along the tonotopic axis. Simultaneous deletion of both MYO7A-C and MYO7A-N abolishes MYO7A immunoreactivity in all hair cells, accompanied by disorganized hair bundles and profound hearing loss. Therefore, we conclude that MYO7A-C and MYO7A-N are the major isoforms in hair cells. We previously reported that IHCs in mice lacking MYO7A-C showed a significant reduction in resting open probability in IHCs, consistent with the proposed role of MYO7A in generating tip-link tension. We now show that deletion of MYO7A-N results in progressive hearing loss and OHC loss. ATPase hydrolysis assays demonstrated that MYO7A-N exhibits significantly lower ATPase activity than MYO7A-C, consistent with a proposed role in maintaining a higher tip-link tension in OHCs. Cryo-EM analysis revealed structural divergences between MYO7A isoforms, with loop-2—a key actomyosin binding interface—showing significant differences that likely underlie their distinct ATPase activities. These structural and biochemical distinctions suggest that MYO7A-N functions as a slower, high-tension motor, specialized for sustaining elevated tip-link tension in OHCs.

Conclusions: In summary, our studies reveal the isoform diversity of MYO7A in the cochlea and highlight their essential roles in tensioning the MET complex. The differences in motor activity between the two isoforms are consistent with their proposed role in fine-tuning the tip-

link tensions, with potential importance for establishing the remarkable frequency range of mammalian hearing.

Taperin Bundles F-Actin at Stereocilia Pivot Points Enabling Optimal Lifelong Mechanosensitivity

8:00 a.m. - 9:00 a.m.

Inna Belyantseva^{*1}, Chang Liu², Abigail Dragich³, Takushi Miyoshi⁴, Sayaka Inagaki¹, Ayesha Imtiaz¹, Risa Tona¹, Karen Sofia Zuluaga-Osorio³, Shadan Hadi³, Elizabeth Wilson¹, Eva Morozko¹, Rafal Olszewski¹, Rizwan Yousaf¹, Yuliya Sokolova¹, Gavin Riordan¹, Andrew Aston¹, Atteeq Rehman¹, Cristina Fenollar Ferrer¹, Jan Wisniewski¹, Shoujun Gu¹, Gowri Nayak⁵, Richard J. Goodyear⁵, Jinan Li², Jocelyn Krey⁶, Katya Krasnopolsky¹, Talah Wafa¹, Rabia Faridi¹, Samuel Adadey¹, Meghan Drummond¹, Benjamin Perrin⁷, Dennis Winkler¹, Matthew Starost⁸, Hui Cheng¹, Tracy Fitzgerald¹, Guy P. Richardson⁵, Lijin Dong⁹, Peter Barr-Gillespie⁶, Michael Hoa¹, Gregory I Frolenkov³, Thomas Friedman¹, Bo Zhao²

¹NIDCD/NIH, ²Indiana University School of Medicine, ³University of Kentucky, ⁴Southern Illinois University School of Medicine, ⁵University of Sussex, ⁶Oregon Hearing Research Center and Vollum Institute, OHSU, ⁷Indiana University Indianapolis, ⁸ORS/NIH, ⁹NEI/NIH

Background: Pathogenic variants of TPRN are associated with nonsyndromic prelingual deafness DFNB79 (Rehman et al., 2010) or progressive hearing loss (Bashir et al., 2013; Li et al., 2010). Taperin protein (TPRN) is located at the tapered base of each stereocilium where it pivots in response to sound (Rehman et al., 2010). TPRN deficiency in mouse results in stereocilia degeneration (Chen et al., 2016; Liu et al., 2018; Men et al., 2019; Qi et al., 2024). However, the exact functions of taperin and molecular mechanisms of stereocilia degeneration in Tprn mutants are unknown.

Methods: We generated two Tprn-deficient mouse models: Tprn^{-/-} has the entire Tprn gene deleted using Crispr/Cas9 and Tprn^{Δ260-749} lacks the sequence encoding residues 260 to 749. ABR and DPOAE were assessed. Whole cell patch clamp recordings evaluated mechano-electrical transduction (MET) in cochlear hair cells. Structural abnormalities in hair cells were investigated with Airyscan confocal and super-resolution STED fluorescence microscopy and various electron microscopy techniques, including FIB-SEM. Finally, the ability of taperin to cross-link F-actin was explored using mouse TPRN heterogeneously expressed in E. coli and purified for biochemical assays.

Results: In wild-type hair cells, native TPRN encircles the F-actin core along stereocilia tapers forming funnel-like patterns like those reported by Qi et al. (2024). TPRN interacts with membrane receptor PTPRQ, connecting stereocilia F-actin to the plasma membrane. Localization of TPRN partially overlaps with TRIOBP-5 and ANKRD24. Overexpression of TPRN in wild-type hair cells results in over-elongation of stereocilia, indicating that TPRN may directly regulate F-actin dynamics. Consistent with this idea, in mice with progressive deafness due to total or partial deletions of Tprn, TRIOBP-5 and ANKRD24 disappear from rootlets of the shortest third row stereocilia beginning at P3, leading to excessive pruning of stereocilia, dysmorphic rootlets and prominent abnormalities in the actin core of stereocilia and rootlets

around stereocilia pivot points. However, MET current before the onset of hearing is unaffected. By P30, these structural abnormalities of stereocilia become accompanied by synaptic rearrangement and excessive accumulation of endosome-like vesicular aggregates in inner hair cells (IHCs) reflecting vesicular trafficking dysfunction along with re-appearance of IHC axo-somatic synapses, indicative of affected MET. This likely exacerbates the progression of hearing loss to profound by two months of age, revealing complex molecular mechanisms underlying deafness due to TPRN deficiency. Biochemical experiments show purified TPRN cross-links F-actin forming flexible bundles with smaller interfilamentous space than F-actin bundles cross-linked by espin isoform 1. Similar whorl-like bent bundle formations are observed in live COS-7 cells. We show that TPRN has multiple actin-binding sites, while its N-terminal portion is essential and sufficient to bundle F-actin.

Conclusions: TPRN bundles F-actin at stereocilia pivot points to reinforce rootlets, stabilizing stereocilia structure and augmenting sustained pivot-point flexibility for optimal mechanosensitivity.

PIP2-Tmie Interactions Drive Mammalian Hair Cell Slow Adaptation Independently of Myosin Motors

9:00 a.m. - 9:15 a.m.

Giusy Caprara¹, Ye-Ri Kim², Sujin Jun¹, Sihan Li³, Un-Kyung Kim⁴, Jung-Bum Shin³, Anthony Peng^{*1}

¹*University of Colorado Anschutz Medical Campus*, ²*University of Colorado*, ³*University of Virginia*, ⁴*Kyungpook National University*

Background: Auditory stimuli are transmitted to the inner ear, eliciting vibrations within the cochlear partition, comprised of hair cells and supporting cells situated atop the basilar membrane and covered by a tectorial membrane. Hair cells serve as auditory mechanoreceptors utilizing apically located hair bundles to transform sound-induced mechanical vibrations into electrical activity, a phenomenon referred to as mechano-transduction. This process is pivotal for cochlear amplification, which underlies exceptional sound level sensitivity, wide dynamic range, and notable frequency discrimination. This mechano-transduction is similar in the vestibular hair cells within the inner ear, which are responsible for balance. With a sustained step-like displacement, the hair-cell receptor current peaks followed by a decrease in the receptor current, termed adaptation. Among these adaptation mechanisms, slow adaptation, characterized by time constants typically ranging from 10-100 milliseconds, was previously hypothesized to operate via a motor model of adaptation. This form of adaptation relies on Ca²⁺ influx through MET channels modulating the attachment of myosin motors along the stereocilia core. Our recent investigations have challenged the motor model of slow adaptation, proposing instead a novel model reliant on the phosphoinositide PIP2.

Methods: We used whole-cell patch clamp electrophysiology coupled with mechanical stimulation with a fluid jet while imaging the hair bundle at 10,000 frames per second to study the slow adaptation process.

Results: We found that the reduction of hair bundle PIP2 levels using PAO results in a reduction of slow adaptation. Previous work by others and us have shown that when the Myo1c motor is

inhibited in vestibular hair cells, slow adaptation is reduced. Strikingly, we could rescue slow adaptation during Myo1c inhibition with exogenous PIP2, indicating that PIP2 is the critical component to slow adaptation, and that the myosin motors may have a secondary role in slow adaptation. Slow adaptation in cochlear hair cells could also be rescued with PIP2 when slow adaptation was reduced with myosin motor inhibition using intracellular sulfate. Our hypothesis is that PIP2 directly modulates the MET channel complex as other ion channels, like the structurally similar TMEM16a, are directly modulated by PIP2. Interestingly, TMIE, an MET channel complex protein, was shown to bind PIP2. Using a point mutation in TMIE that reduces PIP2 binding, we found these hair cells exhibit reduced slow adaptation.

Conclusions: Our results provide the first data describing a new mechanistic model of slow adaptation in mammalian hair cells.

Sensory Transduction Defects Alter Development and Maturation of Auditory Inner Hair Cells

9:15 a.m. - 9:30 a.m.

Thibault Peineau^{*1}, Stephanie Mauriac¹, Lauren Celardo², Benjamin Tibbetts², Jeffrey Holt¹, Brikha Shrestha², Gwenaelle Geleoc¹

¹*Boston Children's Hospital, Harvard Medical School*, ²*Mass Eye and Ear/Harvard Medical School*

Background: We hypothesize that acquisition of mechanosensory transduction plays a pivotal role in the development and maturation of the peripheral auditory system. However, the extent to which alterations in sensory transduction affect downstream pathways and the reversibility of these changes are poorly understood.

Methods: We performed experiments in several mouse models with altered sensory transduction: mice lacking Transmembrane Channel (TMC) proteins one or two (Tmc1 Δ/Δ , Tmc2 Δ/Δ , respectively), mice lacking both proteins (Tmc1 Δ/Δ ; Tmc2 Δ/Δ) and mice carrying recessive mutations in Tmie (Tmiesr/sr, Spinner mice). We assessed electrophysiological properties of inner hair cells (IHCs) before (P12) and after the onset of hearing (P21) to characterize hair cell biophysical profiles and exocytosis. Single-cell transcriptomic profiles were collected from organs of Corti harvested from each mouse model (P24-P28) to determine changes in IHC gene expression. Finally, we performed gene replacement therapy in Tmc1 Δ/Δ mice and assessed recovery of physiological and transcriptional features.

Results: We demonstrate preservation of potassium currents and synaptic properties in Tmc2 Δ/Δ mice. In mice lacking sensory transduction (Tmc1 Δ/Δ , Tmc1 Δ/Δ ; Tmc2 Δ/Δ and Tmiesr/sr mice), we observed alterations in potassium currents, voltage-dependent calcium currents and exocytosis. These changes were associated with alterations in synapse morphology, consistent with previous results. Single cell RNA sequencing revealed different transcriptomic profiles in IHCs of the various mouse models with trends consistent with our physiological assessments. Lastly, we showed that neonatal gene replacement therapy in Tmc1 Δ/Δ mice restored developmental features of hair cell physiology, afferent synapses and gene expression.

Conclusions: The data demonstrate that sensory transduction plays a critical role in the development and maturation of the peripheral auditory system.

Protein-Protein Interactions of TMEM145 in Outer Hair Cell Stereocilia

9:30 a.m. - 9:45 a.m.

Dennis Derstroff¹, Marisa Flook Pereira², Antonia Löhnes³, Sherylanne Newton², Vijay Renigunta⁴, Saskia Hanemaaijer⁵, Carlos Aguilar², Jeffrey R. Holt⁶, Michael R Bowl², Dominik Oliver⁴, Katrin Reimann^{*7}

¹University Hospital Marburg, Philipps-University Marburg, ²UCL Ear Institute, University College London, ³University Hospital Marburg, Philipps-University Marburg, ⁴Institute of Physiology and Pathophysiology, Philipps-Universität Marburg, ⁵Mammalian Genetics Unit, MRC Harwell, ⁶Boston Children's Hospital, Harvard Medical School, ⁷University Hospital Marburg, Philipps-University Marburg

Background: In outer hair cells (OHCs), mechanosensory transduction (MET) relies on physical coupling of the stereocilia bundle to the tectorial membrane (TM). This connection is mediated by tectorial membrane attachment crowns (TM-ACs) at the tips of the tallest stereocilia. Additionally, OHC stereocilia are interconnected by horizontal top connectors (HTC). Both structures are assembled from the same proteins, but their molecular architecture remains incompletely defined. Here, we characterize the interactions of Tmem145, a GPCR-related transmembrane protein, with known TM-AC and HTC components.

Methods: Protein interactions were assessed in-vitro using co-immunoprecipitation, and in cells by pull-down assays (Nano SPD). This in-cell approach is based on intracellular trafficking of bait protein along filopodial actin filaments enforced by association to a myosin motor. Molecular interactions are then quantified by the degree of co-trafficking of fluorescently labelled prey protein to filopodial tips, using confocal microscopy. Interaction interfaces were probed by mutagenesis and deletion of protein domains. Subcellular localization was investigated by super-resolution TauSTED microscopy, while SEM was used to examine hair bundle morphology and TM-AC imprints.

Results: Using CoIP we demonstrate direct physical interactions between TMEM145 and tubby, as well as between TMEM145 and STRC, but not between tubby and STRC, establishing TMEM145 as a molecular bridge linking the intracellular protein tubby with the extracellular protein STRC.

To evaluate direct protein-protein interactions of Tmem145 in live cells we used the nanoscale pulldown technique (NanoSPD). When tubby was co-expressed with Tmem145 fused to GFP-labelled myo10MD, it was significantly enriched in filopodial tips. Enrichment was not observed in the absence of TMEM145 as the bait, indicating binding of tubby to Tmem145. Truncation analyses further suggested potential binding sites mediating the TMEM145–tubby interaction specifically for the intracellular domain of TMEM145.

All three proteins display a ring-like distribution at the tips of the tallest OHC stereocilia, precisely outlining the localization of TM-ACs. Quantitative analysis of ring diameters supported localization of tubby in an innermost layer, an intermediate localization of TMEM145, and the localization of STRC in an outermost layer. Depletion of the lipid co-ligand of tubby, PI(4,5)P2 from the inner leaflet of stereocilia membrane led to dissociation of tubby from the TM-AC complex, supporting its intracellular association to the complex.

Conclusions: Our findings identify Tmem145 as a key organizer of outer hair cell (OHC) stereocilia attachment complexes, bridging intracellular and extracellular components. Through its C-terminal domain, Tmem145 interacts with the intracellular partner tubby, while its extracellular Golgi-like domain anchors the secreted protein STRC. These results uncover a central role for Tmem145 in coordinating protein–protein interactions within TM-ACs as well as HTC. Future work will address the precise binding motifs and assembly principles of these complexes, possible additional components of the complexes, thereby advancing our understanding of OHC stereocilia architecture.

Nherf1 Directs PCDH15-CD2 Trafficking to the Hair Bundle via a Phospho-Dependent Interaction

9:45 a.m. - 10:00 a.m.

Aray Adylkhan^{*1}, Anna Andreeva², Xufeng Qiu³, Alison Lim¹, Phillip Wilson¹, Bao-Tich Nguyen¹, Ulrich Mueller³, Xiaowei Lu⁴

¹*University of Virginia*, ²*Nazarbayev University*, ³*Johns Hopkins University, School of Medicine*,

⁴*University of Virginia School of Medicine*

Background: The architecture of hair bundles (HBs) is crucial for hearing and relies on extracellular links composed of Protocadherin-15 (PCDH15) and Cadherin-23 (CDH23). The CD2 isoform of PCDH15 (CD2 hereafter) is essential for kinociliary links and tip links; its absence during development leads to kinocilium detachment and HB shape and orientation defects, while its absence in mature hair cells (HCs) impairs mechanotransduction (MET) and causes profound deafness. Despite its importance, the molecular mechanisms regulating CD2 trafficking and function in stereocilia are unclear. We hypothesized that CD2 localization to stereocilia is regulated by interactions of its C-terminal PDZ-binding motif (PBM) with PDZ-domain-containing chaperones. This is tested by identifying CD2-interacting proteins and functional analysis of novel CD2-protein interactions in vitro and in vivo.

Methods: To identify CD2 binding partners, we performed a CD2 pull-down assay against P0 mouse brain lysates, followed by mass spectrometry (MS). We confirmed direct binding and determined key residues in CD2 essential for the interaction using in vitro GST pulldown assays and AlphaFold structural modeling. We investigated the physiological relevance of the identified protein-protein interaction (PPI) by analyzing mouse lines carrying mutations that disrupted the PPI, including evaluations of CD2 localization, HB morphology using immunofluorescence, HC MET function using FM1-43 uptake and whole-cell patch-clamp recordings, and ABR hearing thresholds at different stages.

Results: We identified the PDZ-scaffold protein Nherf1 as a CD2 binding partner. In vitro, Nherf1 showed a strong, direct interaction with PCDH15-CD2 and CD1 isoforms. In P0 Nherf1-KO HCs, CD2 localization to stereocilia was significantly reduced and shifted towards the tips, whereas localization of CDH23 was unaffected. Thus, Nherf1 is specifically required for stereocilia trafficking of PCDH15.

We also identified three C-terminal phosphorylation sites in CD2 expressed in heterologous cells. We have generated antibodies that specifically detect phospho-S1771. Importantly, CD2-S1771 phosphorylation was detected in postnatal HBs, suggesting that phospho-regulation of

CD2-Nherf1 interaction is important for CD2 function in vivo. In support, in vitro binding assays demonstrated that a phospho-deficient mutant of CD2 (CD2-4A) has significantly reduced affinity for Nherf1. Moreover, AlphaFold modeling revealed that the 4A mutations disrupt critical hydrogen bonds between the Nherf1 PDZ and CD2 PBM domains.

We next generated corresponding CD2-4A knock-in mice, which phenocopied Nherf1-KO mice. Specifically, CD2-4A HCs at P0 showed reduced CD2 stereocilia localization and mild HB orientation defects. Interestingly, CD2-4A HCs displayed decreased FM1-43 dye uptake, although MET channel currents appeared normal at P5. By one month of age, CD2-4A mice showed high-frequency hearing loss. MET channel recordings at different ages and in different conditions, and ultrastructural analysis of CD2-4A adult HBs are underway.

Conclusions: Disruption of CD2-Nherf1 interaction leads to CD2 stereocilia trafficking defects and compromised MET in adult HCs, with mild effects on HB development.

Podium 9: Auditory Cortex and Thalamus: Human and Animal Studies

8:00 a.m. - 10:00 a.m.

Meeting Room 209ABC, Floor 2

Moderators: Kiril Nourski and Rose Rizzi

Auditory Training Enhances Speech Perception in Noise With Enhanced Cingulo-Opercular Network Activation

8:00 a.m. - 8:15 a.m.

Leeseul Shim¹, Jahee Kim², Gibbeum Kim¹, Hyo-Jeong Lee^{*3}

¹Hearing, Balance and Integrated Neuroscience (HBIN) Lab, Hallym University College of Medicine, ²Hallym University Sacred Heart Hospital, ³Hallym University College of Medicine

Background: The ability to perceive speech-in-noise (SiN) is essential for human social interaction and remains a significant challenge for individuals with hearing loss. Such ability cannot be sufficiently restored by amplification through hearing aids alone but has been shown to improve with auditory training that targets auditory–cognitive functions. Neuroimaging evidence further suggests that speech-in-noise perception is closely associated with higher-order networks supporting auditory attention and cognitive control, such as the cingulo-opercular network (CON). In this study, neurophysiological changes associated with improvement in speech-in-noise performance were evaluated in hearing aid users who underwent auditory training, using functional MRI.

Methods: An fMRI experiment with a SiN task was conducted, and the Korean Matrix Sentence Test (K-MST) served as a behavioral measure. Participants were fifteen adults with moderate to moderately severe hearing loss and sixteen age- and gender-matched normal-hearing controls. The hearing-impaired group was assessed twice, at one month and three months after initial hearing aid fitting, with one subgroup receiving auditory training (HA+AT) and the other using hearing aids only (HAonly). Controls were tested once. Statistical analyses were performed in R, and neuroimaging data were processed with SPM12.

Results: In the normal-hearing controls, the speech-in-noise task elicited significant activation in the bilateral auditory cortices as well as in the cingulo-opercular network (CON), with the latter showing a linear increase in activity as task difficulty increased. Among the hearing-impaired participants, significant improvement in speech-in-noise performance was found in the HA+AT group, whereas no improvement was observed in the HA-only group. In the fMRI results, no group differences were evident at the initial measurement; however, at follow-up a significant group difference and a group $\times$ time interaction were identified in the paracingulate cortex, part of the CON. The interaction reflected a significant increase in activity in the HA+AT group.

Conclusions: We identified that the cingulo-opercular attention network plays a critical role in speech-in-noise processing, with its activity varying according to task difficulty. In hearing aid users, this network was further implicated in the improvement of speech-in-noise perception following auditory training. These findings suggest that auditory training should not be limited to bottom-up auditory approaches, but should also incorporate top-down strategies targeting auditory attention and related cognitive functions.

Invariance to Extrinsic Acoustic Variation is Accomplished by Hierarchical Anatomical and Temporal Computations Selective for Speech-Specific Structure

8:15 a.m. - 8:30 a.m.

Dana Boebinger¹, David Skroll¹, Guoyang Liao², Kirill Nourski³, Matthew Howard⁴, Christopher Garcia³, Thomas Wychowski¹, Webster H. Pilcher¹, Samuel Norman-Haignere^{*2}

¹*University of Rochester Medical Center*, ²*University of Rochester*, ³*The University of Iowa*,

⁴*University of Iowa Hospitals and Clinics*

Background: Coding information in speech is computationally challenging in part because factors extrinsic to the sound source (e.g., reverberation, background sounds) substantially alter the sound's acoustics. Successful communication requires listeners to rapidly encode speech information in a manner that is "invariant" to such acoustic variation. Invariant coding has been studied for decades in the visual system and is thought to be achieved by hierarchical anatomical computations that increase invariance across hierarchically organized brain regions, as well as hierarchical temporal computations that increase invariance across time within a region, partly reflecting recurrent computations. By comparison, less is known about the neural computations that underlie invariant representations of speech in the human auditory cortex. Much of the prior research has been conducted in non-human animal models, which lack speech-specific computations present in human auditory cortex, and non-invasive neuroimaging methods lack the precision needed to measure invariant representations of speech.

Methods: We measured responses to speech presented with many common types of extrinsic acoustic variation using spatiotemporally precise intracranial recordings from throughout the human auditory cortex. We developed a novel paradigm that directly measures the timing and strength of invariance relative to the onset of speech information. The paradigm provides a straightforward, model-free, and time-varying measure of invariance that accounts for the fact that noise in the neural measurements will inevitably cause responses to differ even if the noise-free responses were invariant to acoustic variation. In a separate experiment, we measured

responses to a diverse set of natural sounds without any added acoustic variation, which enabled us to identify speech-selective regions of human non-primary auditory cortex.

Results: We observed neural populations whose responses to speech were nearly completely unaltered by many distinct types of extrinsic acoustic variation (reverberation, background sounds, spectral variation). These neural populations clustered in speech-selective regions of non-primary auditory cortex, suggesting the importance of hierarchical anatomical and speech-specific computations. Invariance also rapidly increased over time in many electrodes, in a manner that could not be explained by generic delays in the neural response, consistent with hierarchical temporal computations. As a consequence, neural responses in speech-selective regions required approximately 200 ms to become strongly invariant to extrinsic acoustic variation.

Conclusions: Collectively, these findings suggest that invariant representations of speech are produced by speech-specific computations that rapidly increase invariance across both time and the auditory cortical hierarchy. The fact that many neural populations become more invariant over time suggests that invariant representations are computed by hierarchical temporal computations that iteratively refine an initial, more acoustically sensitive representation of sound. This type of dynamic process might, for example, reflect recurrent connections within or between regions.

Investigating Human Brain Synaptic Plasticity Through AMPA Receptor PET Imaging in Hearing Loss and Restoration With Cochlear Implantation

8:30 a.m. - 8:45 a.m.

Toshihito Sahara*¹, Akinori Kashio¹, Yasuhiro Arai², Katsuyuki Ishida³, Masahiro Takahashi⁴, Nobuhiro Nishiyama⁵, Hidehiko Takeda⁶, Shujiro Minami⁷, Keisuke Aizawa², Takashi Wada⁸, Hajime Koyama¹, Nobuhiko Oridate², Hiroki Abe⁹, Kenji Kondo¹, Takuya Takahashi⁹

¹*Faculty of Medicine, University of Tokyo*, ²*Yokohama City University Hospital*, ³*Chigasaki Chuo Hospital*, ⁴*International University of Health and Welfare Mita Hospital*, ⁵*Tokyo Medical University Hospital*, ⁶*Toranomon Hospital*, ⁷*National Institute of Sensory Organs, National Hospital Organization Tokyo Medical Center*, ⁸*Yokohama City University Medical Center*, ⁹*Yokohama City University Graduate School of Medicine*

Background: Cortical plasticity in the auditory system could underlie the recovery process after cochlear implantation (CI) for hearing loss. However, the molecular basis of experience-dependent neuronal, and synaptic plasticity in the human brain remains elucidated. AMPA receptors are fundamental molecules of synaptic plasticity, and the amount of AMPA receptors at synapses underlies long-term potentiation (LTP) and long-term depression (LTD), a fundamental mechanism of experience-dependent plasticity such as learning and memory. In this study, we evaluate synaptic plasticity in vivo by visualizing AMPA receptors, using the novel PET tracer [¹¹C]K-2, recently developed in Japan, the only technology to visualize and quantify AMPA receptor in living human brain (Miyazaki et al., *Nature Medicine* 2020) during the recovery of CI. The tracer only depicts AMPA receptors on the cell surface which are the physiologically crucial fraction. The objective of this study was to assess AMPA receptor-mediated synaptic plasticity in the human brain through cross-sectional comparisons with

healthy controls, and longitudinal evaluation before and after cochlear implantation using [¹¹C]K-2 PET.

Methods: This single-arm, multicenter, prospective study enrolled 30 adult CI candidates with acquired bilateral severe-to-profound hearing loss (jRCTs031230617). All participants underwent preoperative [¹¹C]K-2 PET scanning, and 22 completed six-month follow-up imaging after CI surgery. Analyses focused on (1) longitudinal comparison of pre- and postoperative scans, and (2) cross-sectional comparison of preoperative candidates with a cohort of age- and sex-matched healthy controls. Voxel-wise statistical analyses were conducted using SPM12, and regional binding was quantified using SUVR (standardized uptake ratio) with white-matter as a reference (quantitative value of ‘absolute’ AMPA receptors density) and SUVR with whole-brain reference normalization (‘relative’ AMPA receptors density).

Results: In the CI group (63.5±15.7 years), 29 were right-handed and 1 was left-handed, and the surgery side was right in 14 and left in 16 participants. White-matter–referenced analysis showed that, compared with healthy controls, preoperative CI candidates exhibited globally elevated AMPA receptor density. In contrast, longitudinal analysis demonstrated a global decrease from the preoperative to postoperative state. Still, AMPA receptor density in postoperative CI patients tended to remain globally higher than in healthy controls. Whole-brain–referenced analysis revealed postoperative relative increases in AMPA receptor density within the left superior temporal gyrus region, including primary auditory cortex and Wernicke’s area, along with relative decreases in bilateral parietal regions.

Conclusions: This study provides the first in vivo molecular evidence of synaptic plasticity in the human brain after hearing loss and cochlear implantation, revealing dramatic absolute changes in AMPA receptor density and postoperative relative increases within the language-dominant auditory cortex. These findings highlight [¹¹C]K-2 PET as a powerful tool to investigate the molecular mechanisms of auditory plasticity. Overall, these findings underscore the fundamental importance of auditory input for human brain function.

The Nexpo Study: Behavioural, Physiological, and Neuroimaging Measures to Assess the Effects of Noise Exposure on the Auditory Pathway

8:45 a.m. - 9:00 a.m.

Rebecca Dewey*¹, Sofya Koryagina¹, Hannah Guest², Michael Asghar¹, Rebecca Millman², Garreth Prendergast², Susan Francis¹, Christopher Plack²

¹*Sir Peter Mansfield Imaging Centre, University of Nottingham*, ²*Manchester Centre for Audiology and Deafness, The University of Manchester*

Background: The NExpo study aims to determine the effects of noise exposure, relative to those of age and audiometric threshold elevation, on the human auditory nervous system. We hypothesize that measures of auditory function and health will be reduced in noise-exposed individuals. The data collection protocol was presented at the ARO 2024 Midwinter Meeting and has been published.

Methods: Participants: We recruited 200 healthy participants into four groups of n=50 based on age, pure tone audiometry (PTA), and Noise Exposure Structured Interview (NESI) score. Group 1: 18-19 years, low noise exposure, normal hearing; Group 2: 30-50 years, low noise exposure,

normal hearing; Group 3: 30-50 years, high noise exposure, normal hearing; Group 4 30-50 years, high noise exposure, elevated 3-8 kHz PTA.

Data collection: Participants underwent extended high-frequency (EHF) audiometry, speech in noise testing, and measurement of distortion product otoacoustic emissions (DPOAEs), middle ear muscle reflexes (MEMRs), and the electrophysiological auditory brainstem response (ABR). MRI measures included whole-brain anatomical MPAGE data, T1 myelination mapping, and Diffusion Tensor Imaging (DTI), as well as resting-state fMRI of the auditory network, and neurography and Diffusion Tensor Imaging (DTI) of the auditory nerve.

Results: Participant demographics: The mean ($\pm$ stdev) age for Groups 1-4 was 19 ± 1 , 40 ± 6 , 41 ± 6 , and 41 ± 6 years, and NESI scores were 1 ± 2 , 4 ± 4 , 71 ± 68 , and 180 ± 310 units.

Audiological results: Tinnitus Functional Index (TFI) scores significantly differed across groups ($p < 0.001$), with noise-exposed individuals exhibiting significant tinnitus. Group audiometric differences were significant ($p < 0.001$) at 3-6 kHz and EHF. Mean DPOAE amplitude ($p < 0.001$), and speech reception threshold in speech-shaped noise ($p=0.04$) and multi-talker babble ($p < 0.001$) varied significantly with group, while MEMR threshold did not. In chirp-evoked ABRs, wave I amplitude ($p=0.023$), wave V amplitude ($p < 0.001$), wave V latency ($p < 0.001$), and mean inter-peak interval ($p < 0.001$) differed significantly with group (uncorrected for multiple comparisons). Post-hoc tests showed significant differences to be driven by Group 4, with no significant differences between Groups 2 and 3.

MRI results: MPAGE data were quantified using FreeSurfer to estimate group differences in cortical thickness (CT) and grey matter volume (GMV). Our results show a main effect of noise exposure related to decreased CT and GMV in both middle temporal gyrus regions and higher-order cognitive areas, including the frontal, parietal, and temporal lobes. A main effect of hearing loss also related to a more focal reduction in CT and GMV in middle temporal gyrus and superior frontal and inferior parietal regions. Analysis of the remaining MRI measures is underway.

Conclusions: There is no evidence from these data that noise exposure in the absence of threshold elevation affects physiological or behavioral measures of hearing. We aim to determine if MRI measures will facilitate the identification of individuals at risk of neural damage resulting from noise exposure.

A Hierarchically Organized Sound Discrimination Pathway in Auditory Cortex

9:00 a.m. - 9:15 a.m.

Steve Lomber^{*1}, M. Charlotte Kruger¹, Amee Hall², Andres Carrasco², Paul Cornwell³

¹McGill University Faculty of Medicine, ²University of Western Ontario, ³The Pennsylvania State University

Background: Electrophysiological, behavioural, and connectional studies have identified a ventral or “what” processing stream within extrastriate visual cortex of the human, monkey, and cat. This pathway arises in occipital cortex and projects anteroventrally across the temporal lobe. Furthermore, studies have shown that visual areas along this pathway become tuned to increasingly complex visual stimuli. Similarly, in auditory cortex, electrophysiological studies

suggest that areas beyond primary auditory cortex (A1) exhibit a hierarchical organization with responsiveness increasing for more complex acoustic stimuli along the sylvian gyrus of the feline temporal lobe. To examine this idea, we tested the hypothesis that ventral, but not dorsal, areas of the cat's temporal lobe have greater specificity for complex acoustic stimuli.

Methods: We trained four mature cats to discriminate sounds in a two-alternative forced-choice apparatus. The animals concurrently learned to discriminate three classes of sounds: tones, narrow-band bursts, and conspecific vocalizations (“meows”). After training, we implanted cooling loops bilaterally over primary auditory cortex (A1), second auditory cortex (A2), temporal cortex (area T), and insular cortex (area IN) to permit their temporary and reversible deactivation. The animals were then tested while each of the areas was bilaterally or unilaterally deactivated. Presentation of the three classes of stimuli was randomized within each testing session.

Results: With criterion performance of 70% correct on three consecutive days, we identified that conspecific vocalizations were learned the fastest, while tonal discriminations often required twice as much time to master. Bilateral deactivation of A1 resulted in discrimination deficits on all three stimulus classes. Bilateral deactivation of A2 caused deficits only for the narrow-band burst and conspecific vocalization classes. Bilateral deactivation of area T resulted in deficits restricted to the conspecific vocalizations. Bilateral deactivation of area IN did not produce deficits on any of the three stimulus classes tested. Unilateral deactivation of left, but not right, area T caused deficits exclusively during conspecific vocalization discriminations.

Conclusions: The present investigation provides evidence for the lateralization of conspecific vocalization discrimination in the left hemisphere. Overall, specificity for increasingly complex acoustic stimuli was identified along the temporal lobe of the cat. The results of this study indicate a “what” processing pathway in auditory cortex of the cat that arises in primary auditory areas and radiates down the temporal lobe through A2 and into area T.

This work was supported by grants from the Canadian Institutes of Health Research, the Natural Sciences and Engineering Research Council of Canada, and the Canada Foundation for Innovation.

A Topographic Protomap in Subplate Neurons Precedes and Persists Through Onset of Sensory Experience

9:15 a.m. - 9:30 a.m.

Travis Babola*¹, Minzi Chang¹, Rachel Jun¹, Marina Cardoso de Oliveira¹, Jessica Messier², Yunru Chen¹, Lillian Choi¹, Dave Gutierrez¹, Patrick Kanold¹

¹*Johns Hopkins University*, ²*Division of Neonatal-Perinatal Medicine, Johns Hopkins University School of Medicine*,

Background: The establishment of precise sensory maps in the neocortex requires accurate topographic relay of inputs from the thalamus to layer 4 (L4) neurons. Before reaching L4, thalamocortical axons (TCAs) pause within the subplate and form transient connections with subplate neurons (SPNs), a developmentally enlarged population located beneath the cortical plate. Classical ablation studies in cats and rodents suggest that SPNs are essential for guiding TCAs and for the proper formation of cortical maps. However, the functional organization of

SPNs, the nature of their thalamic inputs, and their activity patterns during the critical pre-sensory period remain poorly defined, largely due to a lack of genetic tools that specifically access SPNs. Understanding how SPNs integrate thalamic input and participate in early network activity is critical for defining their role in establishing cortical circuitry.

Methods: To overcome limitations in SPN labeling, we generated a tamoxifen-inducible Cplx3-CreER mouse line, taking advantage of the specific expression of Complexin 3 in SPNs. This model enabled selective genetic access to SPNs in auditory, visual, and somatosensory cortices. We crossed Cplx3-CreER mice with fluorescent and calcium indicator reporter lines to monitor SPN activity and morphology. Widefield and two-photon calcium imaging were used in vivo to assess spontaneous and sensory-evoked responses before and after ear and eye opening. Complementary whole-cell recordings in acute thalamocortical slices characterized intrinsic properties and thalamic inputs to SPNs. Together, these approaches provided a cellular- and circuit-level view of SPN activity across development.

Results: We found that Cplx3-CreER specifically and efficiently labeled SPNs, which persist as L6b neurons into adulthood. Electrophysiological recordings revealed early postnatal maturation of intrinsic properties and demonstrated reliable glutamatergic input from TCAs both before and after ear canal opening. Prior to sensory experience, SPNs exhibited robust spontaneous calcium transients tightly correlated with ascending thalamic activity. These spontaneous events were spatially organized into a protomap aligned with future tonotopic and retinotopic axes. In the auditory cortex, spontaneous organization at P8 resembled future tonotopy, while in visual cortex, SPNs were entrained by propagating retinal waves. Before ear canal opening, SPNs did not respond reliably to sound, but after opening they exhibited frequency-dependent responses with a coarse tonotopic gradient. Compared to L4 neurons, SPN maps were broader and less precise, consistent with a role in establishing cortical organization.

Conclusions: Our findings demonstrate that SPNs receive functional thalamic input, integrate spontaneous peripheral activity, and form an early topographic scaffold prior to sensory experience. After sensory onset, they exhibit coarse but reliable sensory responses. These results identify SPNs as key organizers of early thalamocortical activity, supporting the hypothesis that cortical maps emerge in the subplate before L4 innervation and are subsequently refined by experience-dependent mechanisms.

Auditory Cortical Manifold in Normal and Impaired Hearing

9:30 a.m. - 9:45 a.m.

Satyabrata Parida^{*1}, Jonah Stickney¹, Brad Buran¹, Stephen David¹

¹*Oregon Health and Science University*

Background: Listeners with sensorineural hearing loss (SNHL) exhibit perceptual deficits in everyday communication, which are not fully remediated with modern hearing aids. A key contributing factor to this problem may be central neural plasticity following peripheral hearing loss, which can dramatically affect perception. Studies using simple stimuli (e.g., tones, noise) have revealed changes in the auditory cortex consistent with perceptual deficits. However, because cortical neurons show tremendous heterogeneity in response to natural sounds, it remains unclear how these changes affect the encoding of real-world natural stimuli.

Methods: To comprehensively capture cortical tuning properties across real-world sound statistics, we recorded single-unit activity in the auditory cortex of awake ferrets in response to > 50 hours of natural sounds (pooled across all sessions). Data were from ferrets with normal hearing (N=4, ~3500 neurons) or noise-induced hearing loss or NIHL (N=1, 260 neurons), induced using 2.8–5.6 kHz noise (116 dB SPL, 2 hours). Hearing loss was validated with ABRs.

To characterize cortex-wide tuning properties, we trained a convolutional neural network (CNN) to predict neural responses from sound for each animal. The CNN-based encoding models identified a neural manifold, an efficient, low-dimensional space that is most predictive of cortical population activity. Data were also recorded for an environmental sound corpus (ESC50) to quantify how sound categorization is affected with hearing loss, measured with linear spike-count-based population decoders.

Results: CNN-based models explained over 50% of the explainable variance in neural responses, outperforming previous state-of-the-art encoding models. The manifolds were highly similar between normal-hearing ferrets and captured several key cortical tuning properties, such as spectrotemporal tuning and contrast gain control. In contrast, the NIHL manifold was less similar to normal-hearing manifolds and was lower dimensional, suggesting reduced information processing capacity following hearing loss. Sound categorization performance for the ESC50 dataset was also significantly reduced following hearing loss.

Conclusions: These results establish the neural manifold as a comprehensive, quantifiable framework to characterize the tuning properties that shape cortical responses to real-world sounds within and across individual listeners. These results also provide new insight into how NIHL degrades cortical sound representations and how encoding manifolds capture the underlying plasticity-related changes. This framework opens up important avenues for research to develop new classes of hearing-aid algorithms that mitigate deficits elucidated by degraded manifold representations.

Work supported by: NIH K99DC022330 (SP), R01DC014950 (SD).

Inhibitory Inputs From SST and PV Neurons in the Thalamic Reticular Nucleus Exert Differential Control Over Frequency Tuning in the Auditory Thalamus

9:45 a.m. - 10:00 a.m.

Audrey Drotos^{*1}, Solyman Rolon-Martinez¹, Julie Haas², Maria Geffen¹

¹*University of Pennsylvania Perelman School of Medicine*, ²*Lehigh University*

Background: A primary role of the brain is filtering out irrelevant sensory information from the continuous stream of inputs in our sensory environments. The thalamic reticular nucleus (TRN) is a thin sheet of GABAergic neurons that envelops the thalamus and provides targeted inhibition to thalamic sensory regions, gating which information reaches cortex. The TRN is comprised of multiple types of inhibitory neurons, including somatostatin-positive (SST) and parvalbumin-positive (PV) inhibitory neurons. We recently showed that SST and PV neurons within the auditory TRN provide direct inhibition to distinct anatomical regions of auditory thalamus. In addition, these neurons exhibit differential control of the thalamocortical relay: inhibition of PV neurons can cause facilitation or suppression of MGB neurons, but SST inhibition primarily

results in MGB suppression. However, how these TRN neuron populations specifically exert control over frequency tuning within MGB has not been explored.

Methods: We analyzed the data from in vivo recordings from MGB neurons in SST-cre or PV-cre mice expressing the Cre-dependent inhibitory opsin GtACR in the TRN, which allowed selective inhibition of SST or PV neuron populations, and examined frequency response functions of MGB neurons in both light-on and light-off conditions.

Results: We first selected neurons with good ($R^2 > 0.6$) Gaussian fits to their frequency response functions. We defined the best frequency as μ (mean of Gaussian fit), and frequency tuning width as σ (standard deviation of the Gaussian fit). There were no significant changes in the best frequency of MGB neurons for either PV or SST inactivation. In MGB neurons that were facilitated by PV inactivation, we found an increase in σ , suggesting that inactivating PV neurons broaden tuning. In MGB neurons inhibited by PV inactivation (potentially via a dual inhibitory synapse) we found the opposite effect: σ decreased in the light-on condition, suggesting that inactivating PV drives sharpening of frequency tuning in facilitated neurons. SST inactivation largely resulted in MGB neuronal response suppression, potentially acting via a dual inhibitory synapse. For these neurons, σ decreased, suggesting that without SST inputs, the tuning sharpens in suppressed neurons.

Conclusions: These results suggest that PV and SST neurons exhibit differential control over MGB tuning that is dependent both on TRN cell type and projection pattern within the MGB. This differential control may help TRN neurons to control sound processing depending on the behavioral context, providing mechanisms to both sharpen and widen tuning in an experience-dependent fashion.

Symposium 3: No SOX, No Shoes, No Service: Critical Roles for Sox in the Inner Ear

Chair: Melissa McGovern, *University of Pittsburgh School of Medicine*

Co-Chair: Brad Walters, *University of Mississippi Medical Center*

8:00 a.m. - 10:00 a.m.

Meeting Room 208ABC, Floor 2

Symposium Description: SOX (SRY-related HMG-box) transcription factors play crucial roles in the development, maintenance, and regeneration of tissue systems in many eukaryotic organisms. Sox genes are expressed at all stages of otic development and are present among non-sensory cells throughout life. From the induction of the otic placode, where the rudimentary structure of the inner ear first forms, Sox genes are involved in directing cell fate. Moreover, Sox genes are critical for the appropriate differentiation of cochlear progenitors into hair cell and non-hair cell fates in the developing cochlea and vestibule. Beyond development, loss of function mutations in Sox genes have been shown to disrupt stria function and fluid homeostasis and are linked to human deafness and vestibular dysfunction.

Despite the extensive evidence that Sox genes are not only expressed in the inner ear but also affect proper development and cellular identity, our understanding of how SOX factors regulate cellular identity in the inner ear is still limited. This symposium will discuss current and recent work highlighting the importance of Sox family genes in the inner ear. We will have 6 speakers

who will present work from both vestibular and cochlear organs in mice as well as the Zebrafish lateral line. We are requesting 2 hours for this symposium that will include one 30-minute talk by Amy Kiernan to introduce Sox genes and to review the precedent findings in the inner ear. We will then have five 15 minute talks describing recent and ongoing research investigating Sox genes in the inner ear. Following each talk, we will have ~3 minutes of questions.

SoxC Transcription Factors Shape the Epigenetic Landscape to Establish and Maintain Competence for Hair Cell Generation

Ksenia Gnedeva, *University of Southern California, Keck School of Medicine*

Individual Abstract: Hair cell loss in the adult mammalian cochlea is irreversible and leads to permanent hearing deficits. Many non-mammalian vertebrates, however, can naturally regenerate hair cells in their adult hearing organs via transdifferentiation of supporting cells. Importantly, the transdifferentiation of supporting cells to hair cells can also occur in neonatal mice, showing that mammals still possess the genetic programs to regenerate hair cells yet quickly lose these shortly after birth. In recent work, we uncovered a potential mechanism that explains the ability of non-mammalian vertebrates but not adult mammals to regenerate hair cells. Through cross-species multiome analysis of zebrafish, lizard, and mouse inner ear sensory epithelia, we identified a novel class of supporting cell-specific enhancers associated with key prosensory genes such as *Atoh1* (Wang et al., 2023; Shi et al., 2024). We showed that these enhancers remain accessible but inactive in the adult supporting cells of regenerative vertebrates, reinitiating *Atoh1* expression and converting supporting cells to hair cells following damage. In contrast, while these developmental enhancers remain accessible in supporting cells of the neonatal cochlea, they are rapidly closed and silenced in mice during the first week of life, coinciding with the loss of regenerative capacity. These findings implicate continued accessibility of developmental enhancers for *Atoh1*, and likely other prosensory genes, as a key requirement for persistent regenerative capacity. Further, our work on mouse prosensory development and zebrafish inner ear regeneration converged on the SoxC factors, Sox4 and Sox11, as main candidates for maintaining prosensory enhancer accessibility in supporting cells of regenerative vertebrates. In the prosensory domain of the embryonic mouse inner ear, we have shown that Sox4/11 directly bind and are required for the initial gain of chromatin accessibility of a number of syntenic prosensory enhancers, including ones for *Atoh1* and *Eya1* that are maintained in supporting cells of the adult zebrafish inner ear. SOX is the most enriched binding motif in both mouse and fish prosensory enhancers, and we found that disrupting SOX binding sites in the conserved supporting cell-specific *Atoh1/atoh1a* enhancer eliminates its transgenic activity. In addition, we show that expression of Sox4/11 factors is maintained in supporting cells of the zebrafish but not mouse inner ear. Together, these findings demonstrate the pivotal role of SoxC family members as agents of epigenetic and transcriptional changes necessary for establishing and maintaining competence for hair cell generation in the inner ear.

Sox Transcription Factors Drive Hair Cell Regeneration Through Regeneration-Responsive Enhancers in Zebrafish

Erin Jimenez, *Johns Hopkins University*

Individual Abstract: Background: Damage to mammalian inner ear hair cells is irreversible, resulting in permanent hearing loss or vestibular defects. This lack of regenerative capacity sets mammals apart from most other vertebrates, which can continually produce new hair cells throughout life or regenerate them after trauma. The zebrafish is a non-mammalian vertebrate with a remarkable ability to regenerate hair cells completely. Previously, we reconstructed gene regulatory networks (GRNs) controlling hair cell regeneration by integrating transcriptomic and epigenomic data at single-cell resolution. We identified cell-specific transcription factor (TF) motif patterns in chromatin that opened during regeneration. Correlating emergent enhancer activity with differential gene expression revealed key regulatory networks driving regeneration. Sox-family transcription factors, particularly Sox2, emerged as central factors in initiating regeneration, with chromatin accessibility and gene expression patterns indicating a regenerative enhancer landscape enriched for Sox-binding motifs.

Methods: To identify direct genomic targets of Sox2, we performed CUT and RUN on regenerating adult zebrafish inner ear tissue and integrated these data with scATAC-seq profiles to identify candidate enhancers enriched for Sox2 binding. We then investigated if *sox2*, *sox21a*, *sox4b*, and *sox10*, along with putative regeneration-responsive enhancers containing Sox-binding motifs, were necessary for zebrafish hair cell regeneration by systematically testing function through gene knockouts and enhancer mutagenesis using CRISPR/Cas9. We screened for hair cell regeneration defects using the lateral line as a proxy. Finally, we used fluorescent in situ hybridization to spatially map the expression pattern of *sox* genes in the inner ear.

Results: Sox2 CUT and RUN revealed a network of direct target enhancers during hair cell regeneration. Integration with scATAC-seq identified Sox2-bound loci that overlapped with regions of chromatin accessibility gained only after hair cell injury. Using MIC-Drop, we targeted 48 loci containing either confirmed Sox2 occupancy or predicted Sox-binding motifs and found that 7% of the regeneration enhancers tested were essential for hair cell regeneration. Targeting *sox2*, *sox21a*, and *sox4b* genes showed significant impairment in hair cell regeneration, while *sox10* disruption was lethal. Spatially resolving Sox TFs detected in our scRNA-seq dataset showed their enrichment in supporting cells during regeneration.

Conclusions: Our results identify Sox transcription factors as master regulators of hair cell regeneration in zebrafish. By orchestrating chromatin accessibility and transcriptional reprogramming in supporting cells, Sox factors drive progenitor states essential for regenerative competence. These findings position Sox TFs, particularly Sox2, and their enhancers as key targets for strategies to unlock regenerative potential in the mammalian inner ear.

Single Cell Multiome Analysis Provides New Insights Into Differences Between Type I and Type II Vestibular Hair Cells and Suggests SOX2-Dependent Mechanisms May Play a Role

Jennifer Stone, *University of Washington, Virginia Merrill Bloedel Hearing Research Center*

Individual Abstract: In mammals, type I and II vestibular hair cells have distinct morphologies, molecular markers, physiological properties, and innervation, yet little is known about the genetic regulation of these unique properties. In adult mice, SOX2 expression is confined to type II hair cells. We found that Sox2 deletion from these cells using *Atoh1CreER:Sox2^{f/f}* mice induced a shift toward the type I phenotype along with shedding of bouton afferent terminals and acquisition of full or partial calyceal terminals.

To further investigate the role of SOX2, single nucleus multiome sequencing (snRNAseq and snATACseq, 10x Genomics) was performed on utricles from adult *Atoh1CreER:Rosa26tdTomato:Sox2f/f* mice and *Atoh1CreER:Rosa26tdTomato:Sox2+/+* controls. Transcriptomes were analyzed using Seurat Library for unsupervised clustering and cell type/subtype identification. Selected differentially expressed genes (DEGs) were validated via in situ hybridization chain reaction. Chromatin accessibility data were analyzed using Signac and ArchR. Pseudo-bulk ATACseq data were derived based on transcriptome clusters to analyze cell type/subtype specific gene regulatory elements. Hair cell type-specific enhancer activity was explored using AAV-delivered reporters in utricles in vivo.

snRNAseq analysis identified hundreds of DEGs in type I versus type II hair cells with shared gene ontology linked to synapse structure and function, calcium regulation, and the cytoskeleton. Intriguingly, type II hair cells had enriched expression of genes regulating cell-cell or cell-substrate adhesion and cellular projections or migration. We explored the chromatin status of three DEGs: *Dlk2* (enriched in type II hair cells), *Bmp2* (enriched in type I hair cells), and *Paqr9* (enriched in striolar type I hair cells). We identified putative enhancer sites in these genes and are verifying their cell type-specific expression in vitro. After *Sox2* deletion, type II hair cells shifted their transcriptomic profile toward the type I profile. 109 genes were significantly upregulated in type II hair cells at 1 month after *Sox2* deletion, and 169 genes were upregulated at 8 months. Many upregulated genes were normally enriched in type I hair cells. Conversely, 99 and 97 genes were downregulated in type II hair cells at 1 and 8 months after *Sox2* deletion, respectively. We are exploring the expression and function of genes whose expression is changed after *Sox2* deletion that are strong candidate regulators of hair cell and/or afferent terminal morphology. This approach is revealing novel insights into genes and regulatory elements that may maintain the phenotypes of vestibular hair cells and afferent neural terminals in adult mice.

Sox10 Restricts the Formation of Ectopic Sensory Patches in the Postnatal Mouse Cochlea

Melissa McGovern, *University of Pittsburgh School of Medicine*

Individual Abstract: *Sox10* is a transcription factor that is expressed in the inner ear by embryonic day 9.5 and is expressed in the cochlear duct of mature animals. The loss of *Sox10* was previously linked to a loss of prosensory progenitors, possibly through its direct regulation of the Notch ligand *Jagged1*. Previously, Notch activation through the expression of the Notch1 intracellular domain (N1ICD) in the embryonic cochlea resulted in the formation of ectopic sensory patches in non-sensory regions. This phenotype is restricted to early embryonic stages prior to the differentiation of hair cells in the organ of Corti. Moreover, our preliminary data suggest that the loss of one copy of *Sox10* decreases the threshold for prosensory fate induction by Notch activation of the inner ear.

We investigated whether the loss of a single copy of *Sox10* was sufficient to permit prosensory fate among cochlear cells at birth. We activated Notch signaling in the neonatal cochlea using *Rosa26-LoxP-stop-LoxP-NICD-eGFP* (*Rosa26-NICD*) mice and targeted cells in the cochlear duct with *Sox9-CreER* mice. Finally, we removed one copy of *Sox10* with *Sox10LacZ* mice. We induced Notch activation at postnatal day (P) 0 and collected tissue at P7. In addition, we investigated whether different levels of Notch activation would enhance prosensory fate induction using a Doxycycline inducible model. We activated Notch signaling with TetO-N1ICD and targeted cells of the cochlear duct with *Sox10rtTA*. We activated Notch signaling in

Sox10rtTA:TetO-N1ICD and littermate controls with doxycycline in the diet to nursing mothers from P0 to P7 and doxycycline was injected in pups P1 while cochlea were collected at P7.

We observed ectopic sensory patches containing SOX2+ cells when Notch was activated in combination with Sox10 heterozygosity, but not when Notch was activated alone. We also observed that higher Notch activation increased the number of animals who developed ectopic sensory patches. Prosensory induction characterized by proliferation and Sox2 expression was observed throughout the cochlear duct and foci were observed medial to the organ of Corti that contained ectopic hair cell-like cells.

Our data indicate that Sox10 haploinsufficiency among post-natal cochlear cells permits the formation of ectopic sensory patches following Notch activation. Our findings suggest that SOX10 regulates cell fate among postnatal cells, however, Sox10 is expressed well before and after the first postnatal week. Thus, understanding Sox10's role in cell fate in the inner ear may enhance our understanding of the development, differentiation, and maturation of cochlear cells.

An Introduction to Sox Transcription Factors

Amy Kiernan, *University of Rochester*

Individual Abstract: SOX transcription factors were first identified in 1990 by their founding member, the Sry (Sex-determining Region Y) gene, a sex-determining gene that could specify the male phenotype. Subsequently, related genes were identified based on a shared homology region, the HMG (High Mobility Group) box, and termed SOX, for Sry-related HMG box genes. There are 20 different Sox genes in mice and humans that can be classified into different groups from A to H, based on sequence and structural similarities. In contrast to most other transcriptions factors, structural studies showed that SOX proteins can bind and expand the minor groove of DNA, resulting in a strong bend. The group SoxB1 factor, SOX2, has been of particular interest as it has been shown, along with several other factors, to have the ability to reprogram a somatic cell into an induced pluripotent stem cell (iPSC). In the ear, SOX2 has been identified as an essential factor in the generation of the neurosensory lineage. Members of the SOXC family have also been shown to be required for sensory cell differentiation and regeneration. SOX factors have been identified as 'pioneer' transcription factors, characterized by the ability to bind transcriptionally silent chromatin and recruit other transcriptions factors. This feature may explain why Sox factors have such profound effects on cell fate, including in the neurosensory lineage of the inner ear. It is likely that this pioneering ability imbues some Sox factors with the ability to reprogram differentiated cells, suggesting the tantalizing possibility that these factors can be used to replace depleted cell types in the ear through reprogramming of existing cell types. In this symposium, data will be presented on the roles of different Sox transcription factors in critical cell lineages of the inner ear and future directions will be discussed.

Defining the Role of Soxe Genes During the Development of the Auditory Portion of the Inner Ear

Brigitte Malgrange, *University of Liege*

Individual Abstract: Tightly regulated genetic programs govern the development of the auditory portion of the inner ear. Among the key molecular regulators are the SoxE family transcription factors—Sox8, Sox9, and Sox10—best known for their roles in neural crest development and lineage specification. However, their specific contributions to cochlear development remain incompletely understood. In the developing murine inner ear, we observed that Sox9 and Sox10 are co-expressed in the sensory epithelia beginning at the otic placode stage. As the organ of Corti differentiates, their expression progressively becomes restricted to the supporting cell population.

We employed conditional gene ablation and lineage tracing strategies in mice to elucidate the functional roles of Sox9 and Sox10 during cochlear development. Sox10 was found to be dispensable for otic placode formation but essential for its maintenance. In Sox10-null embryos, the cochlea lacked glial cells, which are critical for providing trophic support to spiral ganglion neurons. Despite this deficit, neuronal survival was preserved, likely due to compensatory upregulation of neurotrophins by the organ of Corti.

In contrast, Sox9 deletion resulted in pronounced defects in cochlear patterning, disrupted sensory cell differentiation, and aberrant neuronal innervation. Transcriptomic profiling further revealed that Sox9 regulates key downstream targets, including *Atoh1* and *Jag1*, implicating it in the modulation of Notch and proneural signaling pathways.

These findings highlight distinct yet complementary roles for Sox9 and Sox10 in orchestrating sensory and neural elements in the cochlea, underscoring their critical involvement in auditory system development.

Podium 10: Patterning and Cellular Specification of the Auditory and Vestibular System

10:30 a.m. - 12:30 p.m.

Ballroom B, Floor 3

Moderators: Angelika Doetzlhofer and Kevin Yu

Outer Hair Cell Innervation by Type II Spiral Ganglion Neurons Depends on Eph/Ephrin and Planar Cell Polarity Cues

10:30 a.m. - 10:45 a.m.

Deborah George¹, Nidhee Seernaum¹, Aileen Cui¹, Maeve Hunt¹, Yifan (Paul) Zhou¹, Shubham Kale², Satish Ghimire¹, Michael Deans³, Thomas Coate^{*1}

¹Georgetown University, ²University of Utah, ³Emory University

Background: Type II spiral ganglion neurons (SGNs) are a fascinating, but poorly understood population of cells. During development, type II SGNs show a dramatic pattern of innervation in the cochlea: they extend past inner hair cells (IHCs), turn toward the cochlear base, then travel a few hundred microns before forming synapses with multiple outer hair cells (OHCs). Previously, Planar cell polarity (PCP) signaling was shown to regulate type II SGN turning. Based on current

research from our lab and others, additional sets of molecules are predicted to facilitate type II SGN turning and other aspects of their development.

Methods: In this study, using genetic sparse labeling, 3D single fiber reconstructions, cochlear cultures, and mouse mutants, we investigated Eph/Ephrin signaling in type II SGN development, as well as possible interactions between Eph/Ephrin and PCP signaling.

Results: In situ hybridization and antibody studies indicate EPHRIN-A3 is expressed by developing supporting cells in the organ of Corti, whereas EPHA7 is expressed by type II SGNs. Similar to loss of Vangl2 (a PCP factor), loss of either *Efna3* or *Epha7* increased the percentage of type II SGNs that turn incorrectly toward the cochlear apex. Type II SGN fibers from both *Efna3* and *Vangl2* knockouts showed altered branching patterns, suggesting these factors also regulate later-stage aspects of OHC innervation. EPHRIN-A3-Fc treatment in vitro increased SGN growth cone collapse at E15.5, but enhanced type II SGN branch number at P0. This indicates EPHRIN-A3 may serve different roles in type II SGN development at different stages. Interestingly, loss of *Vangl2* reduced EPHRIN-A3 membrane distribution on supporting cells, suggesting PCP signaling may act upstream of Eph/Ephrin signaling in this process.

Conclusions: These data indicate Eph/Ephrin signaling helps facilitate type II SGN development and that Eph/Ephrin signaling likely interacts with PCP factors in this process. In the future, we will determine the extent to which PCP and Eph/Ephrin signals facilitate aspects of OHC synapse formation.

Irx3/5 Regulate Vestibular and Cochlear Sensory Patterning in the Mammalian Inner Ear

10:45 a.m. - 11:00 a.m.

Xin Weng^{*1}, Tianli Qin², Yuchen Liu³, Bernice Leung³, Mai Har Sham¹

¹*The Chinese University of Hong Kong*, ²*California Institute of Technology*, ³*University of Hong Kong*

Background: In humans, mutations in *IRX5* are associated with Hamamy syndrome, patients display sensorineural hearing loss, suggesting the potential functions of *Irx* genes during inner ear development. In mammalian inner ear, two main compartments, the vestibule and the cochlea, play distinct sensory roles. However, the mechanisms underlying the formation and separation of these two sensory systems remain elusive. This study aimed to study the roles of *Irx3* and *Irx5* in the vestibular and cochlear patterning.

Methods: We utilized genetically mutated mouse models including *Irx3*LacZ/LacZ (*Irx3*^{-/-}), *Irx5*EGFP/EGFP (*Irx5*^{-/-}), and *Irx3*^{-/-}*Irx5*EGFP/EGFP (*Irx3/5*^{-/-}) to investigate the roles of *Irx3/5*. In addition, to investigate the time window during which the functions of *Irx3/5* is required, *Irx3*f/*fIrx5*EGFP/EGFP, *Emx2*Cre, and *Rosa*CreERT2 mouse models were used to generate temporal conditional knockout mutants. Immunostaining and in situ hybridization were performed on whole-mounted and sectioned inner ear to explore expression patterns. Single-cell transcriptomic sequencing data was generated from cochlea and saccule of wildtype and *Irx3/5*^{-/-} inner ears at E14 followed by analysis.

Results: The inner ear morphogenesis of *Irx3*^{-/-} and *Irx5*^{-/-} is slightly affected, while the *Irx3/5*^{-/-} cochlea represented significant phenotype, including enlarged and shortened cochlear lumen.

We observed that *Irx3* and *Irx5* are broadly expressed in cochlea but are absent from the saccular epithelium.

In *Irx3/5*^{-/-}, *Sox2*⁺ saccular and cochlear sensory domains were fused. Notably, ectopic HCs (hair cells) of vestibular-like stereocilia were formed in the medial edge of the cochlea, occupying cochlear GER (greater epithelial ridge) domain.

Removal of *Irx3/5* before E12.5 using a series of temporal conditional mutants, resulted in the presence of ectopic HCs, resembling the phenotype observed in *Irx3/5*^{-/-} cochlea. However, Tamoxifen was administrated at E13.5 and E14.5, cochlear HCs and the organ of Corti developed normally. These observations suggested that *Irx3/5* function was required within a specific time window for inner ear sensory patterning.

To further investigate the roles of *Irx3/5* in the specification of saccular and cochlear sensory patches, we dissected the saccul and cochlea from E14 wild type and *Irx3/5*^{-/-} inner ears for scRNA-seq analysis. It revealed an increased proportion of saccular sensory cells in the mutants, and an up-regulation of saccular-expressing genes in the mutants. Additionally, we identified a vestibular HC-specific marker, *Tnfrsf25*. In *Irx3/5*^{-/-}, we detected an ectopic expression pattern of *Tnfrsf25* overlapped with the supernumerary *Myo7a*⁺ HCs, indicating that these HCs exhibit vestibular identity.

Conclusions: Our findings revealed that *Irx3* and *Irx5* are essential for regulating vestibular and cochlear patterning. They function to segregate the saccular and cochlear sensory regions, and suppress the vestibular identity in the cochlea.

Promoter Accessibility Accounts for Differential Expression of Many Prominent, but Not Most, Genes in Developing IHCs and OHCs

11:00 a.m. - 11:15 a.m.

Chuan Zhi Foo¹, Anne Duggan¹, Bartom Elizabeth¹, Litao Tao², Neil Segil³, Jaime Garcia-Anoveros^{*4}

¹*Northwestern University*, ²*Creighton University*, ³*University of Southern California*,

⁴*Northwestern University Institute for Neurosciences*

Background: We separately collected developing mouse IHCs and OHCs to identify their mRNAs and chromatin states. We examined their transcriptomes by bulk (full coverage) RNA-seq from six biological replicates each to reveal differences in gene expression. We also examined their chromatin conformation by bulk ATAC-seq from two biological replicates each to reveal open vs closed promoter and enhancer elements. We then compared ATAC-seq with RNA-seq datasets to determine if differential chromatin accessibility can account for differential gene expression. Each biological replicate consists of hair cells pooled from multiple neonatal mice of both sexes.

Methods: We separately collected developing mouse IHCs and OHCs to identify their mRNAs and chromatin states. We examined their transcriptomes by bulk (full coverage) RNA-seq from six biological replicates each to reveal differences in gene expression. We also examined their chromatin conformation by bulk ATAC-seq from two biological replicates each to reveal open vs closed promoter and enhancer elements. We then compared ATAC-seq with RNA-seq

datasets to determine if differential chromatin accessibility can account for differential gene expression. Each biological replicate consists of hair cells pooled from multiple neonatal mice of both sexes.

Results: We found that developing IHCs and OHCs have differentially accessible promoters in some of the most important differentially expressed genes. This includes functional genes whose expression is incipient in neonatal hair cells but will be maintained throughout life, and developmental genes which are only expressed transiently. In addition, we also found that HCs express some unique mRNA isoforms and use novel promoters that are not known in other cell types.

Conclusions: Chromatin accessibility plays an important role in regulating differential gene expression between developing IHCs and OHCs. Moreover, HCs express mRNA isoforms not found in other cell types from novel promoters. Here, we provide a comprehensive resource for the identification of promoters and mRNA isoforms of genes expressed by neonatal IHCs or OHCs, which is publicly-accessible for visualization of any gene of interest at <https://igvviewer.s3.us-east-2.amazonaws.com/index.html>.

Tunability of the Insm1 Mutant Phenotype Reveals a Candidate IHC-Inducing Pathway

11:15 a.m. - 11:30 a.m.

Jemma L. Webber^{*1}, Dominika Rogalska², Alyanna Rodelas², Yingjie Zhou³, Jaime García-Añoveros⁴

¹Creighton University, ²Creighton University School of Medicine, ³Northwestern University, Feinberg School of Medicine, ⁴Feinberg School of Medicine and Hugh Knowles Center for Clinical and Basic Science in Hearing and its Disorders, Northwestern University

Background: Inner hair cells (IHCs) and outer hair cells (OHCs), two types of mechanosensory receptors in the cochlea, are essential for hearing and are distinguished by their unique morphology, molecular function, and specialized patterns of innervation. Damage to either population leads to permanent hearing loss, as neither regenerates. Several transcriptional regulators, including Insm1, Tbx2, and more recently Casz1, have been implicated in specifying IHC versus OHC identity, but the upstream signaling pathways driving these specific fates remain unknown.

Methods: We used Insm1 mutant mice, in which approximately half of OHCs lose their fate markers and transdifferentiate into IHCs, as a model to investigate signaling cues that drive IHC differentiation. Organotypic cochlear explants were cultured from Insm1 mutants at embryonic stages and treated with pharmacological activators or inhibitors of candidate pathways to assess their role in transdifferentiation and IHC maturation.

Results: OHC-to-IHC transdifferentiation in explants closely recapitulated in vivo patterns. We identified a candidate signaling pathway that bidirectionally modulates this conversion: pathway inhibition blocked transdifferentiation, whereas pathway activation enhanced it. Additional loss-of-function experiments in vivo targeting a downstream effector reduced the number of transdifferentiated IHCs, decreased expression of IHC markers (Vglut3, Calb2), and altered nuclear morphology.

Conclusions: These findings demonstrate that the *Insm1* mutant phenotype is modifiable and can be tuned by manipulating candidate pathway activity, supporting the hypothesis that a graded morphogen promotes IHC differentiation. Together, these data identify the candidate pathway as a key regulator of IHC fate and highlight it as a promising target for strategies to selectively regenerate hair cell subtypes.

Spatial Hair Cell Patterning During Zebrafish Utricular Development

11:30 a.m. - 11:45 a.m.

Selina Baeza-Loya^{*1}, David Raible¹

¹*University of Washington*

Background: In zebrafish, like in mammalian species, the vestibular sensory epithelia in the otolith organs are organized into central and peripheral zones, called the striola and extrastriola. Striolar and extrastriolar hair cells drive distinct but parallel vestibular sensory circuits and behaviors, which are functionally matured in the fish's first 5 days. In mammals, it has been shown complementary striolar/extrastriolar patterning depends on differential retinoic acid signaling (rich vs deficient, respectively), but hair cell identity during epithelial patterning in development has not been described in detail. We characterized the spatiotemporal development of hair cell types in the growing zebrafish utricle and assessed factors contributing to maturation.

Methods: To determine perinatal hair cell identity, we use hybridization chain reaction fluorescent in situ hybridization (HCR FISH) probes informed by single-cell RNA sequencing in larval zebrafish against genes found in striolar/extrastriolar hair cells 9 (*cabp1b* and *cabp2b*) and retinoic acid synthesizing (*aldh1a3*) or degrading (*cyp26b1*) enzymes. These fluorescent markers identify cellular spatial patterns in the utricular epithelium. We assessed changes in epithelia in wildtype fish (on *myo6b:GFP* or *myo6b:nlEOS* backgrounds) and in fish with a mechanotransduction (*cdh23*) mutation over the course of development and after pharmacological manipulation of retinoic acid. We then used a computational pipeline for cellular segmentation and image analysis to classify cell types and evaluate spatial organization across fish.

Results: In wildtype utricles, hair cells that develop in the first 48 hours express markers for both striolar and extrastriolar hair cells, indicating a surprising transient intermediate hair cell type which eventually becomes extrastriolar. Hair cells added after 2 days are already specified as striolar or extrastriolar. This change is accompanied by complementary expression of *cyp26b1* in the striolar zone and *aldh1a3* in the extrastriolar zone. In *cdh23* mutant fish, where hair cells do not mechanotransduce, intermediate hair cells never transition. However, complementary patterning of *cyp26b1/aldh1a3* in striolar/extrastriolar zones is normal in *cdh23* mutant utricles. Wildtype fish treated with exogenous retinal, which is converted to retinoic acid by *aldh1a3*, showed a decrease in the relative number of striolar hair cells and an increase in intermediate cell types in wildtype fish. Sputnik fish showed an even greater decrease in striolar hair cells and increase in intermediate types after retinal treatment.

Conclusions: We provide evidence that hair cell maturation, and indirectly normal utricular patterning, is dependent on both mechanotransduction-driven activity and molecular signaling mechanisms. Early hair cells born in the first 2 days begin in a transient state that is neither striolar nor extrastriolar and later acquire extrastriolar characteristics, which is dependent on

functional activity. Hair cells born after 2 days develop as either striolar or extrastriolar, depending on retinoic acid signaling. Disruption of both activity and/or retinoic acid signaling results in a loss of normal patterning.

Prox1 Regulates Trophic Function Between Lateral Supporting Cells and Outer Hair Cells

11:45 a.m. - 12:00 p.m.

Braulio Peguero*¹, Mhamed Grati¹, Rafal Olszewski¹, Michael Hoa¹, Wade W. Chien¹, Matthew Kelley¹

¹*National Institute on Deafness and Other Communication Disorders/ NIDCD*

Background: The organ of Corti (oC) in the mammalian cochlea is an organized cellular mosaic that is required for normal auditory function. Normal development of this complex cellular structure requires temporally and spatially coordinated gene expression. This process includes cellular specification, differentiation, and maturation. While some of the genes required for these processes have been identified, the specific effects of many of these genes remain unclear. For instance, the transcription factor Prospero homeobox 1 (Prox1) is transiently expressed in cells located in the lateral compartment of the oC between E15 and birth when its expression begins to decline. The restricted expression of Prox1 suggests that it may regulate specific aspects of development that are unique to lateral oC cell types.

Methods: Prox1-flox and Fgf20-cre alleles were combined to generate viable Prox1-cKO mice in which Prox1 was deleted from the cochlea beginning at E12. We assessed cochlear function by auditory brainstem response (ABR), distortion product otoacoustic emission (DPOAE) and endocochlear potential (EP) measurements. We used standard immunohistochemical protocols and live imaging assay at different ages to evaluate developmental changes in specific oC cell types. Scanning electron microscopy (SEM) was used to visualize the surface topography and cellular structure of Prox1cKO cochleae. Lastly, we used single cell RNA-sequencing (scRNA-seq) to characterize the transcriptomic changes that occur in response to the deletion of Prox1 in different oC cell types.

Results: Our analyses of the Prox1-cKO mice showed similar changes in cochlea morphology to those reported in previous studies, including a malformed tunnel of Corti and greater number of Deiter cells. Initial development of OHCs appeared normal in Prox1-cKO; however, upon closer examination the OHCs begin to die after P15 and are mostly gone by P21. Extra number of Deiter cells that were found at early ages persist through adulthood. The EP doesn't seem to be perturbed in the absence of the tunnel of Corti. Analyses of scRNA-seq indicated that the transcriptomes of OHCs are largely unchanged in the Prox1-cKO mice. Instead, changes in SC transcriptome suggest a partial change in cell fate for pillar and Deiters' cells in which they appear to express some genes consistent with an inner phalangeal identity.

Conclusions: These results provide evidence for a critical role for Prox1 in normal cochlear development. While Prox1 is expressed in the lateral precursors that will give rise to both OHCs and lateral support cells, OHC development appears largely normal in the absence of Prox1. Instead, deletion of Prox1 appears to lead to a partial change in the differentiation of Pillar and

Deiter cell identities. This change disrupts trophic interactions between these support cells and OHCs, leading to the death of OHCs as a secondary effect of abnormal support cell function.

Beyond Ciliogenesis: Novel Roles of RFX1/3 in Cochlear Hair Cell Survival and Hearing

12:00 p.m. - 12:15 p.m.

Ningjin Wu^{*1}, Kathleen Gwilliam¹, Reza Amanipour¹, Wei Song¹, Beatrice Milon¹, Ran Elkon², Ronna Hertzano¹

¹*National Institute on Deafness and Other Communication Disorders, National Institutes of Health*, ²*Faculty of Medicine and Sagol School of Neuroscience, Tel Aviv University*

Background: RFX transcription factors are best known for their role in ciliogenesis, yet their broader functions in the auditory system remain poorly understood. We recently reported that deletion of Rfx1/3 at embryonic day (E)16 in cochlear hair cells leads to profound hearing loss and rapid outer hair cell (OHC) degeneration at the onset of hearing. Notably, this occurs despite a normal sensory epithelium appearance, indicating a critical role for RFX1/3 in hair cells beyond ciliogenesis. Here we define the temporal window of Rfx1/3 function in cochlear development and uncover their downstream transcriptional networks required for hair cell survival.

Methods: The spatiotemporal pattern of Rfx1/3 expression was recorded across embryonic and postnatal stages using RNAscope. To define the role of RFX1/3 in cochlear hair cells, we generated multiple conditional knockout models: Rfx1/3;Myo15-Cre (deletion at postnatal day (P)0) and Rfx1/3;Prestin-CreERT2 (inducible deletion at P4 or P8). Auditory function was assessed by auditory brainstem response (ABR) and distortion product otoacoustic emissions (DPOAEs) testing, followed by histological analysis. Single-cell RNA sequencing and single-nuclei RNA sequencing of cochlea from P7 and P10 Rfx1/3;Myo15-Cre mice were performed to identify downstream regulatory pathways. In addition, these datasets will be compared with multi-omics data generated from Rfx1/3;Gfi1-Cre mice (deletion at E16).

Results: Rfx1 and Rfx3 are broadly expressed in the cochlea across embryonic and postnatal stages, with enrichment in hair cells relative to other cell types. Deletion of Rfx1/3 specifically in hair cells, followed by functional analyses, revealed a narrow temporal requirement for hearing and hair cell survival between P0 and P8. Deletion at P0 or P4–5 induced hearing loss and outer hair cell (OHC) degeneration comparable to deletion at E16. In contrast, deletion of Rfx1/3 from OHCs at P8 had no effect on hearing or OHC survival. Transcriptomic analysis revealed that beyond structural ciliogenesis genes, RFX1/3 regulate transcriptional programs central to cell survival, mitochondrial homeostasis, oxidative stress resistance, and synaptic signaling, pointing to novel regulatory networks essential for cochlear hair cell maintenance after the onset of hearing.

Conclusions: These findings establish RFX1/3 as critical regulators of cochlear hair cell survival, acting within a narrow postnatal window to safeguard auditory function at the onset of hearing. By uncovering downstream pathways linked to mitochondrial function and synaptic signaling, this work highlights novel RFX transcriptional programs essential in the inner ear

expanding from their known role in ciliogenesis to survival regulation, offering new mechanistic insight into hair cell vulnerability in sensorineural hearing loss.

Exploring the Role of Nedd4-2 Ubiquitin Ligase in Hearing Function

12:15 p.m. - 12:30 p.m.

Ronald Pouyo¹, Enes Spahic¹, Nicolas Thelen¹, Kateryna Shostak¹, Brigitte Malgrange¹, Laurence Delacroix*¹

¹*University of Liege*

Background: Ubiquitination is an essential post-translational modification in which ubiquitin, a small regulatory protein, is covalently attached to substrate proteins. This dynamic process governs protein stability, turnover, subcellular localization, and activity, thereby exerting broad control over cellular homeostasis and function across all tissues. During development and functional maturation, ubiquitination is particularly critical for proper spatial and temporal control of protein networks. Consistent with its importance, mutations in the human gene encoding the E3 ubiquitin ligase NEDD4-2 (Neural precursor cell Expressed Developmentally Downregulated 4-2) have been linked to neurodevelopmental and sensory disorders, including periventricular nodular heterotopia and hearing impairments. Upon Nedd4-2 depletion, brain malformation has been shown to arise from defective neuronal migration around the cerebral ventricles, however, the mechanisms by which Nedd4-2 contribute to hearing loss remain unknown.

Methods: We investigated the role of Nedd4-2 in inner ear organogenesis and auditory function using conditional knockout mouse models. Genetic depletion from the early otocyst was achieved by crossing Nedd4-2Lox/Lox with Foxg1Cre animals, while targeted deletion in sensory progenitors was induced using a tamoxifen-inducible Sox2Cre driver. Auditory function was assessed by auditory brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAEs). Cochlear morphology and cellular integrity were evaluated by scanning electron microscopy and immunofluorescent staining over developmental time points. A yeast two-hybrid screen with an E16.5 mouse inner ear library was employed to identify Nedd4-2 interacting partners. Nedd4-2 was transfected in HEK293 cells, along with candidate substrates, to confirm protein interaction and outcome.

Results: Early otocyst-specific depletion of Nedd4-2 led to severe auditory deficits at hearing onset (P18), with elevated ABR thresholds and reduced DPOAEs across frequencies. Despite normal cochlear morphology at this stage, progressive degeneration of hair cells, spiral ganglion neurons, and stria vascularis was observed in a basal-to-apical gradient from P30 onwards. Depleting Nedd4-2 from sensory progenitors was sufficient to cause significant auditory impairments at young age, and also resulted in massive cochlear degeneration over time. Our yeast two-hybrid screen identified novel Nedd4-2 interactors, including an actin-binding protein localized at tight junctions around hair cells. This protein was degraded upon Nedd4-2 expression in HEK293 cells, and was found to accumulate in Nedd4-2-deficient cochleae at early postnatal stages.

Conclusions: Our findings demonstrate that Nedd4-2 is essential for auditory function and cochlear integrity in mice. Its loss leads to early-onset hearing deficits followed by progressive cochlear degeneration. Identification of a novel actin-binding junctional protein as a potential

Nedd4-2 substrate provides new insight into molecular mechanisms that may underlie hearing loss, although its role in deafness and cochlear pathology requires further investigation.

Podium 11: Hearing Loss: Consequences and Adaptation

10:30 a.m. - 12:30 p.m.

Meeting Room 209ABC, Floor 2

Moderators: Aravaind Parthasarathy and Jack White

ECHO: A Data Resource for Cancer, Hearing, and Outcomes Research

10:30 a.m. - 10:45 a.m.

Katharine Fernandez*¹, Amanda Bonczkowski¹, Abu Chowdhury², Quentin Price¹, Lisa Cunningham¹

¹NIDCD, ²Georgia State University

Background: Cisplatin is a highly effective chemotherapy agent, but it causes permanent hearing loss in up to half of treated patients. However, ototoxicity monitoring is rarely implemented in practice. This lack of baseline and follow-up audiograms has hindered characterization of cisplatin-induced hearing loss in adults. To address this gap, we established the ECHO (Enhancing Cancer Hearing Outcomes) dataset, a multi-institutional resource designed to capture high-quality audiologic and oncologic data to study cisplatin ototoxicity and protective factors.

Methods: We partnered with eight U.S. cancer centers to harmonize and aggregate de-identified audiograms and treatment records from cisplatin-treated adult patients. Pre- and post-cisplatin audiometric thresholds were collected alongside clinical variables including cumulative cisplatin dose, dosing schedule, cancer type, sex, age, and concurrent medications. The harmonized dataset currently includes 1,053 unique patients (74% male; median age 58 years, IQR 49–65) contributing 2,452 audiograms. NCI CTCAE and ASHA criteria were applied where applicable. Cumulative cisplatin exposure ranges 50–750 mg/m². Cancer types were predominantly head and neck (81%), with additional cases from lung, bladder, liver, kidney, and gynecologic cancers. Further harmonization and standardized integration of audiological and oncologic variables is underway for two additional centers, improving and expanding the utility of ECHO for future analyses. We used the ECHO resource to examine the relationship between cisplatin dosing regimen and ototoxicity in patients with head and neck cancer.

Results: This large real-world dataset enables detailed analysis of cisplatin ototoxicity. Dosing protocol significantly influenced hearing outcomes: patients on tri-weekly cisplatin had more severe high-frequency hearing loss than those on weekly dosing, confirming greater ototoxic risk with higher individual dose administration protocols. Additionally, patients taking statins during chemotherapy experienced markedly less hearing loss: concurrent atorvastatin use was associated with an approximately three-fold lower incidence of significant post-cisplatin hearing loss (~10% of patients affected vs 29% without statin). This retrospective finding directly informed a Phase II clinical trial now underway to evaluate atorvastatin as an otoprotective

intervention. The large sample size permits stratified analyses by age, sex, cumulative dose, and statin type – analyses that were previously underpowered in single-center studies.

Conclusions: The ECHO dataset is a unique multi-center audiometric data resource that fills a critical gap in cisplatin ototoxicity research. This resource has already validated known risk factors and predicted new protective factors for cisplatin-induced hearing loss. It clearly demonstrates the power of data sharing in bridging laboratory findings with clinical practice. These insights have direct implications for oncology and otolaryngology – from guiding safer chemotherapy protocols to informing hearing conservation strategies and novel otoprotective trials. ECHO continues to expand via new partnerships and is available upon request as a community resource to drive discovery and improve quality of life for cancer survivors.

Designing a Discriminative, Time-Efficient Diagnostic Battery for Small Arms Fire-Like Noise Exposure

10:45 a.m. - 11:00 a.m.

Meredith Ziliak^{*1}, Jax Marrone², Edward Bartlett²

¹*Purdue University; PULSe - Interdisciplinary Life Sciences*, ²*Weldon School of Biomedical Engineering, Purdue University*

Background: Developing a streamlined stimulus battery capable of identifying and classifying damage from noise induced hearing loss (NIHL) would advance diagnostic precision and treatment planning. As individual audiological profiles can differ according to hearing pathology, protocols would ideally capture these distinctions with minimal time. Small arms fire (SAF) noise exposure is a common source of NIHL linked to inner hair cell synapse loss, elevated auditory brainstem response (ABR) thresholds, and poorer gap detection. Building on this work, we aim to establish a sensitive and time-efficient stimulus arsenal to reveal discriminative assessment types of SAF NIHL.

Methods: F344 rats were bilaterally exposed to SAF-like noise overexposure (OE) (120-126 dBpSPL; n=31) or sham exposure (60 dBpSPL; n=11) levels. DPOAEs and two-channel AEPs were recorded pre-exposure and post-exposure days 7, 14, 28, and 56. AEPs included standard ABRs, fast ABRs (fABRs; multi-tone pip sequences yielding thresholds and morphology 4x faster than standard ABRs), MLRs (click trains with varying interclick intervals from 100 ms to 3 s), dynamic amplitude-modulated sweeps (dAMs; sinusoidal AM frequency continually swept from 12-1500 Hz with either gaussian noise or 8 kHz tone carrier to rapidly determine a modulation transfer function 5x faster with 30x improved spectrotemporal resolution compared to traditional AM stimuli), and speech tokens (rectified and non-rectified male-voiced “Purdue” envelope with noise/tone carriers). Each response was analyzed to characterize changes between exposure groups and time points before comparing across stimulus types to identify the combination most time-efficient and sensitive to functional changes following SAF OE.

Results: SAF OE reduced DP amplitudes and elevated ABR thresholds, particularly 7-14 days post-exposure. ~45% of subjects exhibited click threshold shifts (≥ 10 dB at day 7), while others showed little to no threshold change despite OE. Preliminary analyses indicate fABRs provide threshold information comparable to ABRs with significantly reduced acquisition time. MLR peak amplitude and latency were sensitive to both group and time point. Noise carrier dAMs

showed reduced power at 750-1500 Hz post-exposure whereas tone carriers did not. Speech tokens revealed reduced power at the “P” consonant onset, with variable effects on other phonetic regions. dAMs and speech tokens showed similar temporal tracking between carrier types. Speech EFRs demonstrated greater sensitivity between groups using noise and rectified stimuli.

Conclusions: SAF OE produced heterogeneous functional outcomes, with almost half of subjects showing threshold shifts accompanied by OHC function reduction and altered temporal processing. fABRs and dAMs are emerging as tools for rapid assessment of SAF, while speech tokens may capture ecologically relevant processing changes. Ongoing analyses will identify which diagnostic features and assessments should be utilized in a streamlined stimulus battery tailored for SAF noise overexposure, with the potential to be sensitive enough to distinguish between other types of NIHL.

When Animal Models Don’t Speak Human: Reassessing EFRs as Biomarkers of Cochlear Synaptopathy

11:00 a.m. - 11:15 a.m.

Srikanta Mishra*¹

¹*The University of Texas at Austin*

Background: Experimental noise exposure in animal models has established cochlear synaptopathy as the loss of ribbon synapses between inner hair cells and auditory nerve terminals, despite full recovery of outer hair cell function. However, translation to human populations has been fraught with inconsistency, fueling debate over the validity of proposed biomarkers and contributing to a broader replication crisis in auditory science. Among candidate measures, envelope following responses (EFRs) have been widely promoted as objective indicators of synaptopathy, based on the premise that low-spontaneous-rate auditory nerve fibers exhibit robust temporal envelope phase-locking. Yet, evidence from human studies remains equivocal. The aim of this study was to evaluate the reliability of EFRs as biomarkers of cochlear synaptopathy in humans by combining meta-analytic evidence with experimental data.

Methods: To address this, we conducted a systematic review and meta-analysis of 15 studies (n~850) examining EFRs across tinnitus, noise exposure, and aging. As the highest level of evidence for clinical translation, meta-analysis revealed highly variable outcomes, with no consistent evidence that EFRs reliably capture synaptopathic changes in any category—whether noise-induced, age-related, or tinnitus-associated. These findings underscore the limitations of EFRs as standalone biomarkers and highlight the need for mechanistic clarity. Toward this goal, we tested the contribution of cochlear compressive nonlinearities to EFRs in young adults with normal audiograms (n=39). Distortion product otoacoustic emission input/output functions were used to estimate compression threshold and slope, which were then related to suprathreshold EFR phase-locking.

Results: Meta-analysis showed no significant effects across studies for any categories of synaptopathy. Preliminary results suggest that individual differences in cochlear compression may shape EFR sensitivity to amplitude modulation, complicating their interpretation as direct indices of synaptopathy.

Conclusions: Together, the meta-analytic synthesis and experimental findings converge to show that EFRs lack diagnostic reliability as markers of human cochlear synaptopathy—across noise-induced, age-related, and tinnitus-associated etiologies. This calls into question the possibility that synaptopathy, as demonstrated in animal models, translates directly to humans—a result that is not surprising given the broader observation that more than 90% of behavioral neuroscience findings fail to generalize to human populations.

The Role of Microglia in Sex Differences of Noise-Induced Hearing Loss

11:15 a.m. - 11:30 a.m.

Amelia Schaefer¹, Lucille Papile², Alice Burghard*²

¹*University of Illinois Urbana-Champaign*, ²*University of Connecticut School of Medicine*

Background: Noise exposure is the second leading cause for hearing loss. Recent studies have shown that biological sex plays a role in hearing loss after sound exposure with a greater susceptibility in male subjects. However, the cause for this sex-difference in noise susceptibility remains unknown.

A possible explanation for sex-differences in hearing outcomes after noise exposure is the neuroimmune response, which differs between males and females. Inflammatory responses after noise exposure have been reported from the cochlea up to the auditory cortex and it has been proposed that inflammation contributes to synaptopathy in the cochlea.

Microglia, the resident immune cells in the brain, play an important role in the observed neuroinflammation in the central auditory. Several studies have shown that microglia contribute to sex-differences in neuroinflammation and in responses to injury in the adult brain. To elucidate if sex-differences in microglia activation contribute to sex-differences in noise susceptibility we sound exposed microglia depleted mice of either sex and compared their hearing function to control mice of either sex.

Methods: Mice of both sexes were fed PLX5622 medicated chow for two weeks before the start of the experiments and throughout the rest of the study. This compound is a colony stimulating factor-1 antagonist that leads to the abolishment of microglia after 7 days of consumption. Hearing assessments before and after noise exposure were performed via auditory brainstem response (ABR) and amplitude modulation following response (AMFR) measurements. Animals were awake during the 1 h, 113 dB SPL, narrow-band noise (2 kHz wide, centered at 16 kHz) sound exposure. Animals were sacrificed after the last recording for histological assessments.

Results: We found that microglia depletion has no effects on baseline hearing thresholds in either sex. Four days after noise exposure male mice had worse hearing thresholds than female mice in controls. In microglia depleted animals this sex difference was reduced with microglia depleted female mice showing worse hearing threshold than female control mice. Male mice showed similar hearing thresholds in microglia depleted and control conditions. Two weeks after sound exposure hearing thresholds improved in all animals. However, microglia depleted animals showed no sex-difference in hearing loss, while control animals showed a clear sex difference, with the female having less hearing loss.

Conclusions: Microglia depletion reduced sex differences in noise susceptibility in mice. This was due to a greater noise-susceptibility in microglia depleted female mice compared to controls.

This indicates that female microglia serve a protective role in the auditory system after noise exposure.

Kainic-Acid Induced Cochlear Synaptopathy Alters Neural Adaptation in Budgerigars

11:30 a.m. - 11:45 a.m.

Yingxuan Wang^{*1}, Leslie Gonzales², Kenneth S. Henry²

¹*University of Rochester*, ²*University of Rochester Medical Center*

Background: Neural adaptation is the dynamic adjustment of neural responses to the statistics of the preceding acoustic context. This adjustment enables auditory neurons to operate across a wide range of environmental conditions and may be altered by intrinsic changes like sensory impairments, reflecting either a compensatory mechanism or a form of dysfunction. Cochlear synaptopathy is the loss of synapses between the inner hair cells and auditory-nerve fibers, a common health problem associated with aging and overexposure to loud sound. Recent studies in animal models suggest cochlear synaptopathy may alter temporal dynamics of single-unit neural responses, for example amplifying the onset of the peristimulus-time histogram (PSTH).

Methods: To investigate the temporal effects from a systems-level perspective, we developed a novel paradigm using Poisson click train for fast and reliable measurement of neural adaptation. The random nature of the stimulus timing allows us to measure the relationship between ICI and the amplitude of responses adapted to the prior stimulation. ABRs to Poisson click trains were measured in anesthetized budgerigars before and after induction of cochlear synaptopathy with kainic acid, with 50-70% loss of auditory-nerve synapses. The stimuli consisted of 5-second Poisson click trains with an average presentation rate of 40 clicks/s, presented at 50, 65, and 80 dB peak equivalent SPL. Each click was 0.1 ms in duration, and a total of 6000 clicks were presented with alternating polarities at each sound level.

Results: ABRs to Poisson click trains allowed efficient characterization of the neural adaptation. The adaptation functions of ABR wave I, II and V in budgerigars represent the neural temporal dynamic of auditory nerve (AN), midbrain and cortical processing stages along the auditory pathway. Despite the reduction of the overall amplitude, ABR wave I showed 7% more reduction on short ICIs (< 2 ms) compared to long (> 50 ms) ICIs, specifically at 65 dB SPL. Simulations using a computational model of the AN (Zilany et al., 2014) indicate this finding cooccurs with amplified PSTH onset. ABR wave II exhibited both delayed latency, and altered adaptation function shapes at lower (50, 65 dB) but not higher (80 dB) sound levels, which may be a cascade effect from the AN-level effects.

Conclusions: Our novel paradigm efficiently measures neural adaptation by utilizing the random timing of stimulus presentation. Our ABR measurements show that cochlear synaptopathy strengthens AN adaptation, particularly at moderate, conversational levels, which may lead to delayed temporal processing in the midbrain. These findings of altered neural adaptation along the ascending pathway provide insight into neural mechanisms that potentially underlie hearing difficulties in noisy listening environments caused by cochlear synaptopathy.

This research was supported by grant R01-017519 from NIH NIDCD.

Neuromodulatory Mechanisms Underlying Cortical Neuroplasticity and Perceptual Recovery After Noise-Induced Hearing Loss

11:45 a.m. - 12:00 p.m.

Manoj Kumar^{*1}, Thanos Tzounoupoulos¹

¹*Pittsburgh Hearing Research Center, University of Pittsburgh*

Background: Noise-induced hearing loss (NIHL) induces cell-type-specific plasticity in the primary auditory cortex (A1), which supports perceptual recovery. This plasticity involves coordinated changes among key interneuron populations: recovered sound-evoked activity of parvalbumin-expressing (PV) interneurons contributes to the stability of principal neurons (PNs); the reduced activity of somatostatin-expressing (SOM) interneurons permits recovery of both PN and PV activity; and the recovery of vasoactive intestinal peptide-expressing (VIP) interneurons facilitates PN and PV recovery by inhibiting SOMs. While A1 plasticity after NIHL is generally beneficial for restoring perceptual hearing, it fails to fully compensate for auditory features encoded by precise spike timing, such as temporally modulated noise and speech. Moreover, maladaptive forms of this plasticity may contribute to the emergence of tinnitus and hyperacusis. Despite its importance, the neuromodulatory mechanisms that orchestrate this cell-type-specific, system-level plasticity remain poorly understood. To investigate these mechanisms, we focused on synaptic zinc signaling, a major modulator of synaptic transmission and cortical sensory processing. Understanding the role of synaptic zinc in A1 plasticity has the potential to uncover novel therapeutic targets for enhancing cortical and perceptual recovery, while also mitigating the development of tinnitus and hyperacusis following NIHL.

Methods: We combined cell-type-specific acute and longitudinal two-photon Ca^{2+} imaging in awake mice with a genetically encoded synaptic zinc sensor, operant behavioral assays, and pharmacological manipulations in both wild-type and conditional ZnT3 knockout (cKO) mice lacking synaptic zinc.

Results: Using in vivo wide-field and 2-photon Ca^{2+} imaging in awake mice, we found that pharmacological and conditional deletion of AC synaptic zinc reduced A1 PN sound-evoked responses and impaired perceptual recovery after NIHL, suggesting that cortical synaptic zinc contributes to A1 plasticity and perceptual recovery. Next, we tested whether plasticity in A1 synaptic zinc levels is associated with A1 and perceptual plasticity after NIHL. We performed in vivo wide-field Zn^{2+} imaging in awake mice expressing FRISZ-TM, a genetically encoded far-red transmembrane fluorescent zinc sensor that detects extracellular zinc in response to sound stimuli. One day after NIHL, we observed reduced sound-evoked FRISZ-TM (zinc) responses, suggesting decreased synaptic zinc signaling in A1. Ten days after NIHL, we found significantly increased sound-evoked zinc responses, indicating enhanced synaptic zinc signaling. These findings demonstrate that NIHL induces dynamic, time-dependent plasticity in A1 synaptic zinc levels. To further investigate the mechanistic role of zinc plasticity in neural adaptation after NIHL, we examined its effects on specific neuronal populations over time. Using in vivo Ca^{2+} imaging in awake mice, we found that synaptic zinc plasticity contributes to increased PN sound-evoked responses after NIHL, likely through enhanced SOM inhibition.

Conclusions: Our results establish synaptic zinc as a key neuromodulator of cell-type-specific neuroplasticity in A1 and demonstrate that synaptic zinc signaling is essential for perceptual recovery following NIHL.

Area-Specific Homeostatic Regulation of Cortical Sensitivity of Congenitally Deaf Cats

12:00 p.m. - 12:15 p.m.

Prasandhya A. Yusuf¹, Gideon H.P. Sinaga¹, Stella Clarissa¹, Laurentia Yamin¹, Peter Hubka², Jochen Tillein³, Rüdiger Land⁴, Andrej Kral*⁵

¹*Faulty of Medicine, University of Indonesia*, ²*Medical University Hannover*, ³*MED-EL*,

⁴*Institute of Audioneurotechnology, Hannover Medical School*, ⁵*Hannover Medical School*

Background: Homeostatic regulation stabilizes brain activity, preventing excessive excitation and the cessation of activity in divergent neuronal networks with. It involves synaptic rescaling, resetting of spiking thresholds, and adjustments in excitatory-inhibitory balance. These play a crucial role in the impact of sensory deprivation, such as deafness, on the brain.

Methods: To investigate the impact of prolonged deprivation on such regulation, three animal groups were compared: adult hearing cats stimulated acoustically (HA, n=6), hearing cats stimulated electrically with cochlear implants (CIs) (HE, n=6), and adult congenitally deaf cats (CDCs) stimulated acutely with CIs (CDCs, n=5). Stimuli were presented at the contralateral ear and varied in intensity (10 dB steps for acoustic and 1 dB steps for electric stimulation). The auditory brainstem response (ABR) from the acoustic stimulus and the electric eABR were determined. Subsequently, cortical responses were recorded simultaneously using two Neuronexus multielectrode arrays inserted in the primary auditory cortex (A1) and posterior auditory field (PAF). These encompassed numerous penetrations and several hundred recording sites in each cortical area. Local field potential (LFP) and multi-unit (MU) responses were measured for varying stimulation levels. The ABR and LFP thresholds were determined using statistically significant inter-trial phase coherence. The MU thresholds were determined by a significant increase in activity above the prestimulus interval. Comparisons were non-parametric (paired Wilcoxon rank sum or Wilcoxon-Mann-Whitney test).

Results: Cortical thresholds in PAF were consistently higher than in A1. All animals showed lower mean LFP thresholds compared to MU thresholds, and minimal cortical thresholds were consistently lower than the ABR thresholds. HAs had LFP thresholds 6-12 dB below mean ABR thresholds. In HE, electrical thresholds were lower in eABR (1-2 dB). In CDCs, the opposite was found: significantly higher thresholds for the eABRs (1-2 dB difference). In pairwise comparison, the MU thresholds were in all animal groups nominally higher than LFP thresholds. There was no significant difference in the mean thresholds between A1 and PAF in HA ($p=0.381$). In HE there was a smaller difference of LFP and MU thresholds in PAF compared to A1 ($p=0.039$). In A1 of CDCs, a more similar MU and LFP thresholds than HE was found ($p=1.47 \cdot 10^{-12}$), with a pronounced difference to HE ($p=1.014 \cdot 10^{-7}$). However, in PAF of CDCs, the threshold of units was much higher relative to LFP compared to HE ($p=0.0033$).

Conclusions: While in A1 the neurons became more sensitive to CI stimulation in CDCs, in PAF the neurons became less sensitive. This documents an upregulation of the sensitivity to auditory stimuli in A1, and a downregulation in PAF. This demonstrates a dissociation of the homeostatic regulation between A1 and PAF, supporting their differential role in crossmodal processes.

The Impact of Neonatal Intensive Care Unit Sensory Experience on Rodent Auditory Development

12:15 p.m. - 12:30 p.m.

Todd Mowery*¹, Eran Peci¹, Faith Chukwudinma¹, Noor Zaman¹, Shrinidhi Ramachandran¹

¹*Rutgers University*

Background: According to the World Health Organization, ~15 million children are born prematurely each year. Many of these infants end up spending days to weeks in a neonatal intensive care unit (NICU). Infants who are born prematurely are often exposed to noise and light levels that affect their auditory and visual development. Children often have long-term impairments in cognition, visuospatial processing, hearing, and language. OSHA regulations consider noise levels under 85 dB SPL safe for indefinite exposure; however, the infant may be sensitive to noise below this level especially during the critical periods of auditory development.

Methods: To test this, we have developed a rodent model of NICU exposure to light and sound using the Mongolian gerbil (*Meriones unguiculatus*), which has a low-frequency human-like audiogram and is altricial. Postnatal day 11 animals housed with their breeding parents had their eyes and ears opened prematurely or sham opened, after which they were exposed to the NICU-like sensory environment (24 hour light cycle (400 lumen), and 75 (day)/45 (night) dB SPL broadband noise) throughout the gerbil's cortical critical period of auditory development (P11 to P18). Control animals were reared in the same box, with no manipulation and the normal colony environment (12/12 light dark cycle, ambient room noise). Auditory status was assessed in neonates (P19), juveniles (P35), and adults (P86) with ABR and DPOAE recordings. Once adults, all animals were trained on a two-tone auditory discrimination task to determine the presence of any learning or performance impairments. A noise masker was then introduced (50, 75, 100, and 125%). Behavioral performance on noise masker trials were correlated with ABR Wave 1 amplitudes and latencies and DPOAE thresholds to identify animals with possible synaptopathy.

Results: NICU reared animals showed behavioral impairments and changes to auditory status (increased thresholds) compared to control animals.

Conclusions: Our preliminary results suggest that this animal model could provide researchers with an ethical way to explore the long-term effects of NICU exposure on the preterm infant's brain development.

Special Session: Auditory Periphery to Cortex: Research Advances and Career Journeys

10:30 a.m. - 12:30 p.m.

Meeting Room 208ABC, Floor 2

Auditory Periphery to Cortex: Research Advances and Career Journeys

Elinette Albino

Celebrating Hispanic and Latin Scientists: Research Advances and Career Journeys

Michele Insanally*¹

¹*University of Pittsburgh School of Medicine*

Individual Abstract: This session aims to increase the visibility of underrepresented Hispanic and Latinx scientists in the field of Otolaryngology and will include participants both locally from Puerto Rico and from the United States. Hispanics and Latinos are largely underrepresented in the field of Otolaryngology at all career stages from students to postdocs to faculty with only 4.1% Latino full professors in Otolaryngology (Watkins et al., 2024; Solis and Valdez et al 2023). This session will highlight the scientific achievements and career trajectories of Hispanic and Latinx researchers in Otolaryngology, with a focus on increasing visibility and fostering community among underrepresented groups. The panelists will highlight both their research contributions to the field of Otolaryngology as well as career trajectories. Panelists will include seven scientists from different career stages spanning postdoc to senior faculty. The panelists research topics will span a broad range from the auditory periphery to cortex as will their career stage. Topics will include auditory hair cell biology, genetics of hearing loss, head and neck cancer, auditory neuroscience, and health disparities in Hispanic and Latinx populations. Each speaker will present their research, and reflect on their unique career paths, challenges, and opportunities as Hispanic and Latinx scientists. The session will conclude with a moderated discussion and networking opportunity, designed to inspire trainees and facilitate collaborations across geographic boundaries and career-stages.

Impact of Resting Mechanotransduction Current on Stereocilia Actin Dynamics in Mammalian Auditory Hair Cells

A. Catalina Velez-Ortega*¹, Manuela Gomez-Giraldo², Juliana Castro Jimenez²

¹*University of Kentucky*, ²*University of Kentucky, College of Medicine*

Individual Abstract: Background: The mechanosensitivity of inner ear hair cells depends on microvilli-like projections – termed stereocilia – supported by parallel actin filaments. In mammalian hair cells, actin filaments are remarkably stable along the length of a stereocilium, except at a small tip region that exhibits continuous actin turnover. We previously discovered that stereocilia harboring mechano-electrical transduction (MET) channels retract after blockage of the calcium influx through these channels – which are partially open at rest – while the neighboring tallest non-transducing stereocilia in the same hair bundle are not affected. Once the resting MET current is restored, transducing stereocilia regrow. Our preliminary data indicate that the MET-dependent shortening of stereocilia exceeds the tip region of active actin turnover. Lastly, stereocilia of cochlear hair cells are supported by two different actin isoforms (β and γ), which exhibit different polymerization and depolymerization dynamics in the presence of calcium. Here, we are evaluating the extent to which changes in the resting MET current alter the active turnover and composition of the actin cytoskeleton in auditory stereocilia.

Methods: We used tubocurarine to block MET channel currents in isolated neonate cochlear explants from mice. We visualized changes to (a) the incorporation of β -actin via fluorescence recovery after photobleaching using hair cells expressing β -actin-eGFP and (b) the β/γ actin ratio in C57Bl/6 wild-type hair cells using isoform-specific antibodies. Changes were evaluated along the length of individual stereocilia using confocal microscopy.

Results: Our results show that, in inner hair cells, the zone of active actin incorporation in stereocilia progressively becomes smaller as cells mature during the first postnatal week. In addition, the blockage of MET channels decreases both the size of this zone and the rate of actin incorporation in stereocilia from transducing (shorter) as well as non-transducing (tallest) rows. Our preliminary results with immunolabeling against β - and γ -actin indicate that the blockage of MET channels leads to a decrease in the β/γ actin ratio at the tips of transducing stereocilia.

Conclusions: Altogether, our results indicate that the resting MET current in cochlear hair cells is essential to maintain the appropriate size, rate, and composition of the zone of active actin remodeling at the tips of stereocilia. In the presence of MET deficits, it is plausible that actin dynamics shift towards a scenario that is unfavorable for the lifelong maintenance of stereocilia.

Targeted Cell Interconversions Reveal Inner Hair Cell Control of Organ of Corti Cytoarchitecture

Ignacio Garcia-Gomez¹, Jemma L. Webber¹, Berta Soria Izquierdo¹, John C. Clancy¹, Anne Duggan¹, Yingjie Zhou¹, Charles P. Murphey¹, Trevor D.M. Harriman¹, Jianghong Wang¹, Mary Ann Cheatham¹, Jaime Garcia-Anoveros^{*2}

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Individual Abstract: The mammalian organ of Corti is a precisely intercalated mosaic of two types of mechanosensory hair cells and six types of supporting cells (SCs) arranged in eleven parallel rows. Differentially specialized SCs surround inner (IHC) and outer (OHC) hair cells. To elucidate the developmental roles of IHCs and OHCs in the formation, differentiation, and assembly of the various SCs, we genetically switched HC identities at several developmental stages. In addition, we generated mice lacking IHCs. We find that IHCs promote or induce the: (1) differentiation of one type of outer compartment supporting cell (Outer Pillar or OPC) at the expense of another (Dieters' 1 and 2, but not 3), so that each completely and exclusively populates separate rows; (2) high packing density, but not identity, of Inner Pillar Cells (IPCs), so that they form a continuous wall that lines the medial side of the tunnel of Corti; (3) embryonic formation and (4) cytoplasmic attraction of adjacent Inner Phalangeal Cells (IPhCs), so that they tightly envelop IHCs. The longer-range effects of IHCs on outer and inner pillar cells are caused by their transient developmental production of FGF8 ligands, but the effects of IHCs on the immediately adjacent IPhCs are not FGF8-dependent and must involve another signaling mechanism. Hence, developing IHCs dictate major aspects of SC identity and distribution to assemble the complex organ of Corti.

GPSM2-Dependent Regulation of Actin Dynamics and Stereocilia Integrity in Mature Hair Cells

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Individual Abstract: GPSM2 is a critical component of the elongation complex (EC), a group of proteins that drive hair bundle growth and patterning during development. Recent work showed that all EC proteins persist at row 1 stereocilia tips in mature hair cells (Hartig et al., 2024). In adult hair cells, GPSM2 depletion reduces accumulation of other EC proteins at tips and causes a progressive collapse in stereocilia height (~0.5-0.6 μm) matching the tip compartment defined as the actin turnover region. This suggests EC proteins may regulate actin dynamics to maintain dimensions of the tip compartment and preserve hair bundle architecture in mature hair cells under mechanical strain.

We used Gfi1CreERT and tamoxifen delivery to temporally control Gpsm2 inactivation in hair cells. Cochleae were harvested 6 months after inactivation at 6 weeks of age, doubling the chase period from our previous study (3 months). We also inactivated Gpsm2 at 6 months and analyzed hair cells 3 months later. Confocal microscopy was used to quantify hair cell survival, row 1 stereocilia height in inner hair cells, and EC protein enrichment. To track actin dynamics in vivo, we generated a new mouse model expressing HaloTag-ACTG1 upon Cre activation. Crossing this model with Gfi1CreERT; Gpsm2 mutants enabled labeling of newly incorporated actin at tips post inactivation. Stereocilia tip remodeling was further examined using FIB-SEM.

Six months after Gpsm2 inactivation, stereocilia height and EC protein levels were reduced, but not significantly more than after 3 months (Hartig et al., 2024). This suggests that the tip compartment depending on EC proteins is largely depleted by ~3 months already while shafts remain stable. Inactivation at 6 months led to slightly milder height reduction but ongoing analyses suggest hair cell loss and occasional bundle degeneration at the cochlear apex, phenotypes absent in earlier inactivation schemes. Loss of GPSM2 at 6 weeks progressively reduced the amount of new actin (HaloTag-ACTG1) incorporated at tips 4, 6, and 12 weeks later, formally linking EC protein loss to impaired actin turnover. FIB-SEM analysis confirmed a disrupted row 1 stereocilia morphology, including abnormally rounded tips and a reduced electron-dense tip compartment.

Loss of EC proteins in young adult hair cells impairs actin turnover at row 1 stereocilia tips, leading to a progressive remodeling and collapse in size of the tip compartment complete by ~3-4 months. Mild auditory dysfunction observed 3 months post inactivation (Hartig et al., 2024) is thus not expected to worsen. At later stages, GPSM2 loss might further impact stereocilia and occasionally compromise the core as well. We propose that EC proteins not only drive stereocilia elongation during development but also fine-tune actin dynamics at row 1 stereocilia tips to maintain hair bundle integrity throughout life.

Genetic and Audiological Profiles of Hearing Loss in Puerto Rico

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Individual Abstract: Understanding the genetic causes of hearing loss (HL) in Puerto Rico is essential given that approximately 7% of the population experiences significant HL—exceeding mainland U.S. averages. However, the underrepresentation of Puerto Rican individuals in genetic research limits accurate diagnoses and equitable care. This study aims to investigate the genetic and audiological landscape of childhood-onset HL in Puerto Rican families by integrating genomic sequencing, detailed phenotyping, and functional analyses. Through exome and genome sequencing of affected individuals and their parents, we seek to identify population-specific variants that contribute to HL onset and progression. Comprehensive audiological and vestibular assessments are conducted using standardized protocols to correlate genetic findings with clinical presentation. Functional validation in cellular and animal models further elucidates the biological impact of prioritized variants. This approach will help establish a foundational variant database for this population, improve diagnostic precision, and inform personalized treatment strategies. Ultimately, this work aims to reduce health disparities, enhance early detection, and contribute to global efforts to understand HL in diverse and underrepresented populations.

Mechanisms of Serotonergic Signaling in the Auditory Midbrain

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Individual Abstract: The inferior colliculus (IC) is the midbrain hub of the central auditory pathway that processes and integrates many auditory and non-auditory inputs. The IC receives a major serotonergic input from the dorsal raphe nuclei. Serotonin differentially modulate how IC neurons respond to sound. However, because of the diversity of neuron types and complex circuitry in the IC, the mechanisms by which serotonin leads to changes in auditory responses are unclear. We previously identified that neuropeptide Y (NPY) expression is a marker for a class of IC GABAergic neurons while the majority of glutamatergic neurons express the NPY Y1 receptor (Y1R neurons). We hypothesized that serotonin differentially modulates the excitability of NPY and Y1R neurons. To determine the subtypes of serotonergic receptors expressed by NPY and Y1R neurons, we performed fluorescent in situ hybridization in IC brain slices of mice. We used probes against *Npy*, *Vglut2*, and *Htr1a*, *Htr1b*, *Htr2a*, and *Htr2c*. Next, to determine how serotonin modulates the excitability of NPY and Y1R neurons, we performed whole-cell patch clamp recordings combined with serotonin puff application targeted to either NPY (identified in the NPY-hrGFP mouse) or Y1R (identified in the Y1RxAi14 mouse) neurons. Selective antagonists were bath applied to determine the receptors mediating the serotonergic effect. Since many regions that project to the IC express serotonergic receptors, we next used optogenetics to determine how serotonin modulates defined presynaptic inputs to IC neurons. Our data show that 32% of *Vglut2* neurons express *Htr1a*, and 38.2% of *Vglut2* neurons express *Htr1b*. Fewer *Vglut2* neurons express the excitatory serotonergic receptors *Htr2a* (1.1%) and *Htr2c* (14%). *Npy* neurons express either *Htr2a* (45.3%) and/or *Htr2c* (39.2%) receptors. Few *Npy* neurons expressed the *Htr1a* (2%) and *Htr1b* (1%). In agreement, our electrophysiology data show that NPY neurons are depolarized by serotonin by activation of 5-HT_{2A/C} receptors, while Y1R neurons are hyperpolarized by activation of the 5-HT_{1A} receptor. Optogenetics experiments suggest that serotonin decreases the strength of local IC circuits but enhances the strength of contralateral IC circuits. In addition, we find that serotonin can either enhance or diminish the strength of descending cortical projections. Our results suggest that serotonin

signaling favors inhibition in the IC by inhibiting the Y1R and exciting NPY neurons. Future studies will determine whether the effects of serotonin are changed in auditory processing disorders such as tinnitus and age-related hearing loss.

Dynamics of Auditory Cortical Neural Activity in Response to Natural Sounds

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Individual Abstract: Research in the Jaramillo laboratory focuses on the neural circuits responsible for auditory cognition. Our goal is to understand how the brain assigns meaning to sounds, how it attends to or ignores auditory stimuli, and how expectation influences sound perception. In this talk, I will present work characterizing the dynamics and geometrical structure of population activity in the auditory cortex during the presentation of natural sounds. We collected datasets containing hundreds of simultaneously recorded neurons from distinct regions of the mouse auditory cortex. Analyses of these datasets have revealed potential mechanisms that explain a counterintuitive perceptual phenomenon where discrimination accuracy between sound samples decreases for longer-duration samples if the sounds are distinct instances of the same acoustic category.

Poster Session III with Coffee

1:30 p.m. - 3:00 p.m.

Ballroom A, Floor 3

Due to the policies of the Puerto Rico Convention Center, the Poster Hall will be available to attendees between the hours of 7AM – 11PM (local time), with a 1- hour break every day between 11AM and 12 PM (Noon) to facilitate setup for the next poster session. This is an appreciated extension of their normal permitted hours. ePosters are available on the conference website and the ARO MWM mobile app.

M1. Interareal and Interlaminar Differences in Sound Envelope Encoding in Core and Parabelt Auditory Cortex

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Background: Sound envelope fluctuations are a key sound feature that drives the perception of complex stimuli such as species-specific vocalizations and music. Many previous studies have gained insight into how sound envelope encoding differs across the auditory pathway by recording neuronal responses to click trains and sinusoidally amplitude modulated (AM) tones and noise in nonhuman animals. These studies document a hierarchical progression across the pathway, generally characterized by a decrease in the temporal precision of AM encoding as the pathway is ascended. In primary auditory cortex, there are also parallel differences in AM

encoding across cortical layers, with the extragranular layers preferring slower AM relative to the thalamorecipient granular layer. In human and nonhuman primates, additional interhemispheric differences have been observed; namely, the left hemisphere exhibits enhanced temporal encoding relative to the right hemisphere. The NHP model provides an opportunity to understand what circuit mechanisms generate these transformations in encoding by characterizing AM encoding in different cortical circuits within area, and across the cortical hierarchy.

Methods: To address this, here we report the encoding of AM signals as a function of cortical layer and hemisphere in NHP core and parabelt auditory cortex. We recorded concurrent synaptic and multiunit spiking activity using linear array multielectrodes positioned across cortical layers. AM noise and click trains (1.6-200 Hz) were presented while NHPs passively listened. We derived measures of AM frequency classification from the neuronal responses using linear discriminant analysis.

Results: Core AC exhibited enhanced AM classification relative to the parabelt, across all layers. Sites in core AC typically encoded all AM frequencies (1.6-200 Hz) with high (> 90%) accuracy, while sites in the parabelt typically encoded a subset of lower (~1.6-25 Hz) frequencies; often via rapid, synchronized fluctuations in the local neuronal population. Across both areas, the granular and infragranular layers displayed enhanced AM encoding relative to the supragranular layers. Both areas displayed enhanced AM encoding in the left hemisphere, restricted to the supragranular layers.

Conclusions: These results suggest a complex interplay of area, cortical layer, and hemisphere is at work in the NHP auditory cortical hierarchy, and a potential supragranular layer origin of previously documented left-hemisphere dominance in temporal encoding.

M2. Cingulate Cortex Activity is Modulated During an Effortful Task in Gerbils

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Background: When sensory perception occurs under challenging acoustic conditions (e.g., noisy background), our stimulus sensitivity can remain surprisingly unaffected. One plausible neural mechanism involves the recruitment of a top-down neural pathway, from the cingulate-to-auditory cortex, that selectively facilitates auditory processing (Anbuhl et al., 2025). Here, we asked how gerbil cingulate cortex (Cg) neuron activity is modulated when animals engage in an effortful listening paradigm.

Methods: To assess auditory effort, an amplitude modulation (AM) rate discrimination task was used and stimulus parameters (AM rate, sound duration) were varied to adjust the difficulty of listening conditions. Using an appetitive Go-Nogo paradigm, adult gerbils (n=6) were trained to discriminate between “Go” stimuli (range of AM rates: 4.5-12 Hz; broadband noise carrier, 100% depth) and a “Nogo” AM stimulus (4 Hz). Trials were clustered into ‘Easy’ or ‘Hard’ blocks, where the sound duration was 1s or 0.25s, respectively. AM rate discrimination thresholds were determined from psychometric functions. To determine whether Cg neural activity is modulated during effortful listening, chronic electrode arrays were implanted into the Cg1 of behaviorally-trained animals, and wireless recordings from Cg1 neurons were obtained

during task performance. Neural firing rates were computed across stimulus block type (Easy, Hard) and within trials to assess whether Cg activity is selectively modulated with task difficulty. Behavior sessions were video recorded and tracking of animal movements was quantified using SLEAP, a framework for pose tracking via deep learning. Animal tracking data were aligned to behavior trials to extract movement-related metrics (e.g., position, velocity) within each trial. We then examined the relationship between psychometric performance, neural activity, and pose tracking metrics.

Results: Cg1 neurons (n=600) were recorded from gerbils (n=6) across 36 awake-behaving psychometric sessions during which stimuli were delivered in a block design (i.e., 40 easy trials, 40 hard trials). To test whether Cg1 activity was selectively modulated during specific time windows, a block-level analysis of firing rate was conducted within discrete trial epochs (trial initiation, unmodulated stimulus, modulated stimulus, and post-stimulus period). The first two epochs, trial initiation and unmodulated stimulus, yielded average firing rates that were greater for Easy trials across all stimulus types (Go, Nogo). The remaining epochs, modulated stimulus and post-stimulus period, yielded greater firing rates for Hard trials especially for 4 Hz AM (Nogo) and 4.5 Hz AM (the most difficult Go stimulus often associated with a Miss). Analysis of video recordings are currently underway to determine whether pose metrics can be correlated with task difficulty and with elevated Cg activity observed during the AM and post-stimulus period.

Conclusions: Taken together, the results suggest Cg1 neurons are recruited during hard stimulus conditions and are selectively modulated during trial epochs that may be associated with elevated auditory effort (i.e., stimulus, post-stimulus).

M3. Chemogenetic Modulation of Prefrontal Cortex Influences Auditory Cortex Responses and Event-Related Potentials in a Schizophrenia Rat Model

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Background: Research on schizophrenia (SCZ) has shown that cognitive deficits stem partly from an impaired excitatory-inhibitory balance in the prefrontal (PFC) and auditory cortex (AC). Mismatch negativity (MMN), altered in SCZ, arises from AC and PFC where sound patterns, and contextual changes are processed sequentially. Predictive coding theory posit that AC sends bottom-up, ascending sensory signals to the PFC which in turn sends top-down, descending predictions to AC. Thus, top-down predictions modulate responses to expected stimuli, while unanticipated stimuli generate error signals that update the internal model. Pharmacological studies in humans and animal models have shown that MMN reduction in SCZ likely reflects NMDAR hypofunction. Using the chemogenetics technique, we manipulate the mPFC-AC pathway to study how top-down predictions control auditory processing in an animal model of SCZ.

Methods: We implanted Long-Evans female rats with ECoG arrays placed over AC-L, AC-R and PFC-R. We injected a retrograde inhibitory virus (AAVrg-hSyn-hM4D(Gi)-mCherry) into AC-L, and we implanted cannula guides above PFC-L. After recovery, we recorded from awake animals, either without any infusion or an infusion of C21 (5 mg/mL for mPFC-AC inhibition) or saline (sham; 0.8 μ L). We first carried out the recordings in a control condition, then alternated one week with 8 days of MK-801 (0.1 mg/Kg, IP), followed by one week of washout, and the same for MK-801 (0.2 mg/Kg). Auditory stimuli consisted of a classical oddball paradigm using a frequency pair of 11.313 and 16 KHz.

Results: In the control condition, deviant response amplitude increased in the AC-L during mPFC-AC inhibition, but not in the sham infusion. The deviant responses of the AC-L decreased with lower MK-801 dose and increased with the higher dose, but resulting in an abnormal response. We also studied how event-related potential components behaved as a function of MK-801 dosage. Thus, the 0.2 mg/kg dose resulted in a distinct increase of the N1 amplitude while the 0.1 mg/kg dose increased only a marginally compared to the control recording, although these did not reach statistical significance. By contrast, P1 remains unchanged.

Conclusions: Our results show that NMDAR dysfunction induces an alteration of the AC LFP and on the event-related potential components. The C21 infusion into PFC-L increased the amplitude of the AC-L deviant response, an effect that was not present during saline infusion. This suggests that the absence of a top-down PFC predictions, the prediction error increases in AC.

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M4. Layer 6 Corticothalamic Neurons Show Attentional-Gain Responses During a Tone-In-Noise Detection Task

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Background: Neuronal activity adapts to behavioral context, optimizing performance. Although previous studies have shown that layers (L) 2/3 in the auditory cortex show attentional gain when mice are engaged in a detection task, how this modulation is achieved is unknown. Previous studies have reported the presence of noise-invariant neurons in upper cortical layers, and that the amplitude of responses and bandwidth decrease as SNR decreases. Since L6 corticothalamic (CT) neurons play an important role in modulating cortico-thalamo-cortical activity, we hypothesize that this modulation could sharpen the tuning curves and increase the gain of neurons in L2/3 and thalamic nuclei, thereby improving the perception of sound in environments with high background noise, for example. However, it remains unclear whether L6 CT neurons contribute to the mechanisms that improve performance in such conditions. Here, we aim to investigate how L6 CT neurons respond during behavior and whether L6 CT neurons could enhance the perception of target tones embedded in a noisy background.

Methods: We trained adult mice of the Ntsr1-Cre (B6.FVB(Cg)-Tg(Ntsr1-cre)GN220Gsat/Mmucd) line on a fixed hearing background (B6.CAST-Cdh23Ahl+/Kjn) crossed with a reporter line expressing (B6.Cg-Igs7tm162.1(tetO-GCaMP6s,CAG-tTA2)Hze/J) on a tone-in-noise detection task. Constant broadband white noise (50 dB SPL) was presented together with a one-second duration tone of 11.5 kHz (30 to 70 dB SPL), to obtain signal-to-noise ratios (SNRs) of -20 to +20 dB SPL. The mouse had to turn a wheel 25° degrees in either direction to receive a water reward. Mice were then imaged under 2-photon microscopy while performing the task. The pupil diameter was also recorded to assess the animal's attentional state during the task. Passive imaging sessions were conducted after the behavioral sessions to obtain frequency response areas for each neuron.

Results: Our preliminary findings indicate that pure-tone-responsive L6 CT neurons increase their response gain when mice successfully detect a tone embedded in a noisy background, compared to when they do not. This enhancement in response gain appears to become stronger as the SNR decreases.

Conclusions: Our preliminary results are consistent with our hypothesis for a role of L6 CT neurons in hearing in noisy environments. The observed increase in gain of L6 CT neurons at lower SNRs for hit trials in comparison to miss trials suggests that task difficulty and possibly the state of attention may be driving their responses when hearing target tones becomes more challenging.

M5. Morphometry and Nav 1.6 Expression Changes in Cochlear Ranvier Nodes of Ageing C57BL/6N and CBA/J Mice

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Background: Age-related hearing loss (ARHL) is not only caused by hair cell degeneration, but also changes in the auditory nerve, including synaptic loss and impaired neural conduction. Spiral ganglion neurons (SGNs) depend on efficient action potential generation at the hemi node and propagation via Ranvier nodes (RVN), mainly triggered by the sodium channel Nav 1.6. Alterations in node morphometry or Nav 1.6 expression may disrupt this signalling. As SGNs comprise diverse subpopulations with distinct functional and molecular properties, their vulnerability to ageing may differ among humans and mouse strains. Investigating these changes offers insight into the mechanisms underlying ARHL.

Methods: The present work investigates age-related changes in morphometry and Nav 1.6 immunoreactivity of RVN in the auditory system of the mouse to detect temporal changes across the lifespan of the two mouse strains, CBA/J and C57BL/6N. Immunostaining of Nav 1.6 was performed in 1 to 19 months old animals with an automatic immunostainer and quantified with Image J. RVN morphometrics as well as semiquantification of RVN immunoreactivity were done taking into account tonotopic position and auditory path in the cochlea.

Results: A clear tonotopic gradient of Nav 1.6 intensity was evident in both strains, with higher levels in the basal turn, indicating functional specialization along the cochlear axis. Results further show strain-specific age-related changes in RVN morphometry and Nav 1.6 expression. While CBA mice lose small calibre fibers and median RVN length increases, B6 mice show a degradation of big myelinated axons with a reduction in median node diameter. A decrease in

Nav 1.6 immunostaining in middle-aged B6 mice with a subsequent compensation to young mice level in older animals may be explained by an upregulation of Nav 1.6 production after early loss. This Nav 1.6 expression profile perfectly fits to the early hearing deficits in these mice. In CBA mice we do not see such a pattern that matches with ABR data, so hearing deficits in the apical turn do not correspond to changes in Nav 1.6 expression levels.

Conclusions: While B6 mice lose more big calibre nerve fibres with high spontaneous firing rates, CBA mice show the opposite with a pronounced loss of small calibre axons with a low spontaneous firing rate with increasing age. Both results in a loss of the dynamic range of hearing sensation. Nav 1.6 changes affect mainly B6 mice that may contribute to ARHL in this mouse strain.

M6. Auditory Function During the Menopausal Transition in the Accelerated Ovarian Failure (AOF) Mouse Model: Evidence From Young Adult Female CBA/CaJ Mice

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Background: Menopause-related declines in estrogen are believed to contribute to age-related hearing loss (presbycusis). While estrogen and other female sex hormones are known to play important roles in maintaining auditory function, the specific mechanisms by which hormonal loss—independent of aging—affects hearing remain poorly understood. To isolate the effects of ovarian hormone depletion from those of aging, this study evaluated auditory function in young adult female CBA/CaJ mice using the 4-vinylcyclohexene diepoxide (VCD)-induced Accelerated Ovarian Failure (AOF) model. Peripheral and central auditory function was assessed throughout progressive ovarian hormone loss using auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs)

Methods: Female CBA/CaJ mice (n = 14; 2 months old) received daily intraperitoneal injections of VCD; 160 mg/kg/day for 15 consecutive days to induce ovarian failure. Age-matched control mice received equivalent volumes of vehicle. An additional group of ovariectomized (OVX) mice was included to validate the postmenopausal phenotype induced by VCD. Ovarian failure was confirmed through histological follicle counts and serum hormone analyses. Auditory function was evaluated by measuring ABR thresholds and DPOAE amplitudes and thresholds at baseline (pre-treatment) and across three defined stages of menopause: pre-menopause (< 25 days post-treatment), peri-menopause (50–75 days), and post-menopause (> 129 days post-treatment). Cochlear cryosections from post-menopausal VCD-treated mice and controls were stained with a mitochondrial dye and imaged using confocal microscopy for immunohistochemistry (IHC) analysis.

Results: By 129 days post-treatment, visual assessment of VCD-treated mice demonstrated persistent acyclic diestrus, consistent with ovarian failure. Histological analysis of H and E-stained ovarian sections revealed a progressive decline in primordial and primary follicles over

time, while corpora lutea remained preserved. Serum estradiol (E2) levels significantly decreased by day 24, reaching their nadir by day 129. In parallel, follicle-stimulating hormone (FSH) levels began to rise from day 52 onward. ABR testing showed significantly elevated thresholds in VCD-treated mice at days 52 and 129, especially at high frequencies. No threshold changes were observed at day 24. OVX mice similarly developed elevated ABR thresholds four months post-surgery. DPOAE data revealed reduced amplitudes and threshold shifts at low and high frequencies on days 52 and 129 in VCD-treated mice; mid-frequency responses remained stable. Control mice maintained consistent auditory function throughout. Mitochondrial dye was detected in the spiral ganglion, stria vascularis, and organ of corti in both VCD and control cochleae; however, mitochondrial signal was reduced in the postmenopausal VCD group vs. control non-treatment group.

Conclusions: VCD treatment effectively induced accelerated ovarian failure in young CBA/CaJ mice, mimicking key hormonal changes of the menopausal transition. Declining estrogen levels were associated with progressive auditory deficits, potentially influenced by mitochondrial function. These findings support the anticipated effects of the VCD-induced AOF on female hormonal levels which are associated with auditory function and strengthen the evidence linking estrogen deficiency to presbycusis.

M7. Auditory Novelty Detection Deficits in an Alzheimer's Disease Rat Model: Behavioral and Electrophysiological Evidence

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Background: Age-related hearing loss is a widespread disability that impairs communication and social participation in the elderly. Recent studies highlight hearing loss as a significant risk factor for dementia, including Alzheimer's disease (AD; Griffiths et al., 2020). Deviance detection is a fundamental element of the predictive coding framework. This theory posits that the brain compares incoming sounds with expected patterns and identifies deviations from predictions. Peripheral accuracy declines with aging, increasing reliance on predictions. Dementia further impairs prediction reliability, as temporo-parietal dysfunction reduces auditory adaptation. Thus, aging and dementia alter predictive processes cognitively and behaviorally. We investigated how AD pathology affects deviance detection using TgF344-AD rats, a transgenic model with hallmark AD lesions.

Methods: We trained 40 rats, aged 16 to 20 months (TgF344-AD and controls, both sexes) to discriminate deviant tones in an oddball paradigm. Animals learned to nosepoke when a deviant sound occurred and were rewarded with food pellets. We used two frequency sets (4.8–6.7 and 8.0–11.3 kHz). We tested discrimination across interstimulus intervals (1.5–4.0 s), frequency contrasts (0.5, 1.00, 1.25 octaves), and deviant probabilities (10–30%). We also applied a many-deviant paradigm. We quantified behavioral performance using d' . After training, we recorded neuronal activity in the auditory cortex with 64-channel probes, using paradigms adapted to neuronal response ranges (250 ms ISI). We included predictive coding controls: oddball

ascending/descending, cascade ascending/descending, and many-standards. We analyzed firing rates and predictive coding indices (iMM, iPE, iRS).

Results: TgF344-AD rats required more sessions than controls to learn the task and achieve stable performance. They showed lower d' , slower latencies, and reduced sensitivity to small frequency contrasts in the many-deviant paradigm. Males generally outperformed females. Longer ISIs improved performance in all groups. Control animals detected higher frequency contrasts better, whereas TgF344-AD animals performed relatively better at lower contrasts in the many-deviant task. Electrophysiological recordings in auditory cortex revealed attenuated responses to deviant sounds in TgF344-AD rats. Predictive coding indices were also altered: iMM was reduced, reflecting weaker deviance encoding, while iPE and iRS showed disrupted balance between prediction and adaptation.

Conclusions: AD pathology in TgF344-AD rats impairs auditory novelty detection at behavioral and neuronal levels. Animals needed more training, showed poorer discrimination and slower latencies, and recordings revealed reduced deviant responses and mismatch indices. These results demonstrate disrupted predictive processing and validate the TgF344-AD rat as a model for deviance detection deficits.

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M8. Evaluating Auditory Reflex Measurements: Interactions Between MEMR, MOCR, and Methodological Considerations for Research

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Background: The middle-ear muscle reflex (MEMR) and the medial olivocochlear reflex (MOCR) are emerging tools for assessing peripheral auditory function, including hidden hearing loss. However, as with other auditory measurements, the reliability of these tools may be affected by interactions between hidden auditory deficits and outer hair cell (OHC) integrity. Also the influence of MEMR on MOCR measurements remains unclear, since both reflexes are evoked and measured in a similar way. This study evaluates MEMR and MOCR measurements in participants with audiometrically normal-hearing ears, focusing on age-related effects and subtle auditory deficits. In addition, we address methodological considerations relevant for implementing these reflex measures in research.

Methods: This project combines two complementary parts:

- Part 1 assessed age-related effects and MEMR-MOCR interactions. Twenty young (18-35 y/o), normal-hearing participants (≤ 20 dB HL) and twenty older (45-65 y/o), normal-hearing participants were tested using wideband click-evoked MEMR and MOCR in ipsilateral and contralateral configurations. MOCR was measured both with an elicitor noise of 60 (standard) and 75 dB SPL to evaluate potential MEMR contamination.

- Part 2 piloted the effects of subtle auditory deficits on reflex measurements. Ten young normal-hearing controls, five participants with unilateral mild hearing loss (≤ 50 dB HL), and five normal-hearing participants with unilateral tinnitus underwent MEMR and MOCR testing in ipsilateral, contralateral, and bilateral configurations.

Results: Both MEMR and MOCR were decreased in the older test-group, consistent with prior reports. The data of part 1 also reveals that MEMR can affect MOCR estimates, but only at higher stimulus levels, since MEMR typically evokes at higher intensities than the MOCR. Further analyses will be conducted to evaluate whether the effects of MEMR can be reduced in MOCR analyses.

Preliminary results of part 2 suggest that contralateral and bilateral reflex responses are sensitive to subtle hearing deficits. The effects of OHC damage on ipsi- and contralateral MEMR and MOCR measurements will be further evaluated and discussed at the conference. These pilot findings highlight the methodological factors that influence measurement reliability and interpretation.

Conclusions: Wideband click-evoked MEMR and MOCR measurements can be used to study peripheral afferent and efferent function, but interpretation requires careful consideration of age, OHC status, and pathway interactions. By evaluating the effects of age and peripheral deficits within the same framework, this study provides guidance for optimizing reflex protocols in future studies of hidden hearing loss, tinnitus, and other central gain-related conditions.

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M9. A Dual-Pathway Model of Fall Risk: The Mediating Role of Balance

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Background: Falls represent a major public health crisis for older adults and are a leading cause of morbidity and mortality. Postural stability is a complex process that relies on the interaction among auditory, cognitive, and vascular systems. Deficits across these upstream systems are identified as fall risk factors. These factors are often studied in isolation and the mechanistic pathways that link the collective influence to falls remain poorly understood. In this study, we propose that upstream deficits, such as hearing problems, reduced cognitive performance, or poor vascular health, do not cause falls directly but rather converge to impair balance. This study tests the hypothesis that balance acts as the critical mediator, translating these diverse upstream deficits into functional fall risk.

Methods: We analyzed cross-sectional data from 4,805 community-dwelling adults aged 65 or older in the National Health and Aging Trends Study (NHATS) Round 13. The outcome variable was a summated fall-risk score. Predictors included self-reported hearing ability, cognitive function, mental health, and chronic vascular conditions. The mediators were two complementary measures of balance: a subjective self-report of a balance problem and an objective score from the balance subset of the Short Physical Performance Battery (SPPB). We used survey-weighted multiple regression to identify direct predictors and mediation analyses to test for indirect effects through balance.

Results: The analysis revealed two distinct pathways to fall risk. First, a direct pathway was observed where greater self-report balance problems were the strongest predictor of fall risk ($\beta = 0.53$, $p < .001$). Additional direct predictors included lower objective balance scores ($\beta = -0.08$, $p < .001$), symptoms of depression ($\beta = 0.05$, $p < .001$) and anxiety ($\beta = 0.04$, $p = .001$), as well as a history of heart disease ($\beta = 0.07$, $p = .02$). Second, an indirect pathway was identified where poorer hearing ability, lower cognitive scores, and the presence of hypertension each independently predicted worse balance (both subjective and objective). These balance deficits, in turn, strongly mediated the relationship with fall risk, converging on balance impairment as the key mechanism linking these factors to increased fall risk.

Conclusions: Our findings identify the pivotal role of balance in linking deficits in hearing, cognition, and vascular health to fall risk. For the science and practice of otolaryngology, this reframes hearing and vestibular disorders not merely as sensory deficits prevalent in older adults, but as critical contributors to postural instability. The key takeaway message is that hearing and vestibular functions play a central role in multidisciplinary fall-prevention management, as balance represents the common pathway where the upstream diverse risks can manifest. Future research should employ prospective designs to investigate how multi-modal interventions can target this pathway and reduce falls.

M10. Delayed Synaptic Aging in the Cochlea of Long-Lived Naked Mole Rats (Heterocephalus Glaber)

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Background: Age-related hearing loss (ARHL) is one of the most common sensory impairments in humans, often associated with progressive loss of cochlear ribbon synapses. These synaptic changes can precede hair cell and neuronal loss and have been described in several mammalian models, including rodents and humans. The naked mole rat (*Heterocephalus glaber*) is a unique model for studying cochlear aging. Despite its elevated auditory thresholds at young ages—likely reflecting their subterranean lifestyle—naked mole rats (NMR) rely heavily on vocal communication within their social groups. Given their lifespan (up to 31 years) and resistance to cancer and age-associated diseases, NMR provide an opportunity to explore how cochlear synaptic integrity is preserved across aging.

Methods: Cochleae were obtained from naked mole rats aged 4–15 years. Temporal bones were isolated and fixed in 4% paraformaldehyde, decalcified, and immunostained for Prestin (hair cell marker) and CtBP2 (presynaptic ribbons). Confocal microscopy was used to image the cochlea, and IMARIS software was used for quantification of inner and outer hair cells as well as ribbon synapses along the cochlear length. Counts were normalized to cochlear region and age group for comparison.

Results: Hair cell integrity remained stable across the lifespan examined. Confocal imaging revealed no major structural changes from 4 to 12 years of age, and both inner and outer hair cell counts were preserved even in 15-year-old animals. Ribbon synapse counts showed a gradual decline with age, but the reduction did not reach statistical significance. Compared to other rodent models, where substantial synapse loss occurs early in life, naked mole rats displayed a markedly delayed onset of synaptic decline.

Conclusions: Our results show that inner and outer hair cell and ribbon density are remarkably stable with age in naked mole rats. These results indicate that cochlear aging is markedly delayed in naked mole rats, providing new avenues to uncover protective mechanisms that may inform strategies to prevent or slow age-related hearing loss in humans.

M11. Frequency-Specific Mapping of Auditory Nerve Fibers: Investigating G-Ratio in Serial Sections

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Background: The cochlea encodes sound via a precise tonotopic organization, where specific frequencies map to distinct locations along its length, ranging from 1kHz to 100kHz in mice. To achieve frequency-specific analysis on thin plastic sections, we generated a three-dimensional frequency map using high resolution microCT datasets and Greenwood function. Given the minimal anatomical variation in inbred mouse strains, this serves as a universal reference for a precise frequency localization. Age-related auditory nerve degeneration impacts cochlear implant outcomes, with frequency-specific patterns varying across strains, leading to selective loss and preservation of nerve fiber populations that influence hearing function.

Methods: By integrating frequency-specific auditory brainstem response (ABR) audiometry with precise frequency mapping, we correlate physiological data with nerve fiber morphometry. Manual segmentation of the basilar membrane at the inner hair cells (IHCs) level enabled spatial mapping of nerve fibers into an “atlas” dataset, allowing the registration of the thin sections within the microCT dataset for an exact frequency correlated morphological analysis. The software-based segmentation and automated evaluation of the g-ratio using deep learning algorithms will provide an objective assessment of nerve fiber integrity across different frequency regions.

Results: By correlating ABR parameters, including hearing thresholds and latencies between young vs aged mice, we aim to refine our understanding of frequency-specific neural degeneration.

Conclusions: This insights into cochlear pathology will inform strategies for optimizing cochlear implant electrode placement and stimulation paradigms, ultimately improving clinical outcomes by tailoring interventions to preserve and enhance neural function at critical frequency regions, ensuring better auditory performance for cochlear implant users.

M12. Effects of Aging and Hearing Loss on Neural Tracking of Linguistic Features in Rapid Speech

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Background: Aging and hearing loss can greatly impact the ability to understand speech, especially in difficult listening conditions. While age-related changes in the brain's temporal dynamics are known to affect speech processing, the specific impact on linguistic representations, beyond acoustic sensory processing, is not fully understood. This study investigates how aging and hearing loss influence the neural encoding of both sensory and linguistic information in time-compressed speech, an ecologically valid signal that mimics challenging listening environments.

Methods: A total of 94 participants from three distinct groups were recruited: young adults with normal hearing (YNH), older adults with normal hearing (ONH), and older adults with hearing impairment (OHI). All participants completed behavioral testing to determine time-compression thresholds for 50% keyword recognition of rapid speech. Also collected were electroencephalography (EEG) recordings to measure cortical envelope tracking of continuous speech in both time-compressed and unprocessed conditions. The data will be analyzed using a Temporal Response Function (TRF) framework, which will allow us to model hierarchical neural responses from early acoustic features up to higher-order linguistic units like phonemes, words, and phrases.

Results: Behavioral results showed a significant difference in performance between age groups, with YNH participants demonstrating higher overall keyword recognition compared to both ONH and OHI groups. This finding suggests a decline in rapid speech processing with age and hearing loss. We hypothesize that this behavioral deficit will be reflected in altered neural encoding patterns. The EEG analysis will reveal if the previously reported exaggerated cortical tracking responses in older adults are present across all representational levels, from acoustic to linguistic, and how time-compressed speech specifically affects this phenomenon. An acoustic signal that is uninformative due to its presentation rate should disrupt cortical tracking to the greatest extent at the highest levels in linguistic representation: linguistic TRFs for the ONH group will begin to resemble the OHI group in the time-compressed condition.

Conclusions: Our findings will provide new insights into how aging affects the neural encoding of speech at various hierarchical levels, from early sensory processing to complex linguistic representations. The results will highlight the specific challenges older adults face in processing rapid speech and may reveal compensatory neural mechanisms that do not fully restore speech perception. These findings may inform rehabilitative strategies for age-related hearing loss. Key takeaways will include evidence for a cascade of processing deficits in older adults, emphasizing that a listener's ability to understand speech goes beyond simply processing the auditory signal.

M13. SPI-1005 Improves High Frequency Hearing in Meniere's Disease Patients in a Multi-Center, Phase 3, Randomized, Placebo-Controlled Trial

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Background: Meniere's disease (MD) is characterized by episodic vertigo or dizziness, fluctuating low-frequency hearing loss, and intermittent or constant tinnitus. Adults are typically

40-65 years of age at diagnosis and can progress to severe-to-profound hearing loss and intractable tinnitus in the affected ear. MD is typically unilateral but can become bilateral in 25-33% of patients as they age. Age-related hearing loss (ARHL) is characterized by progressive bilateral loss of high-frequency hearing and speech discrimination in older adults (> 50 yo) and may be accelerated by prior noise and ototoxin exposure. SPI-1005, an oral capsule containing ebselen, an investigational new drug with unique anti-inflammatory properties (GPx1 mimic and inducer), was tested in a Phase 3 Randomized Controlled Trial (RCT) with Open-Label Extension (OLE) in MD patients with active symptoms (NCT04677972). SPI-1005 treatment showed clinically relevant improvements in two pre-specified efficacy endpoints involving low-frequency hearing and speech discrimination over placebo. Here, we present post-hoc analyses involving high-frequency hearing and speech discrimination characteristic of ARHL.

Methods: Adults (18-75 yo) with definite MD were enrolled at 11 US sites and were randomized to 28-days of SPI-1005 400 mg or placebo BID. Pure-tone audiometry (PTA) and Words-in-Noise (WIN) were assessed at baseline, 28, 56, and 84-days. Compliant participants entered OLE and received up to 12-months SPI-1005 400 mg BID and were reassessed every 3-months. Improvements in high-frequency PTA (HFPTA) and WIN scores in either ear from baseline were: ≥ 10 dB at two adjacent high-frequencies (4-kHz and 6-kHz, or 6-kHz and 8-kHz) and ≥ 4 -words in WIN score. Subjects were also stratified by age (≥ 50 yo) and sex.

Results: 191 Per-Protocol subjects (mean age 55.5; 103 males/88 females) were analyzed; 141 were ≥ 50 yo (mean age 60.8; 71 males/70 females). During RCT, HFPTA improvements were 18/95 SPI-1005-treated (18.9%) vs 7/96 placebo-treated (7.3%), $p=0.0190$. WIN improvements were 40/95 SPI-1005-treated (42.1%) vs 26/96 placebo-treated (27.1%), $p=0.0336$. In ≥ 50 yo, HFPTA improvements were 14/65 SPI-1005-treated (21.5%) vs 4/76 placebo-treated (5.3%), $p=0.0049$. WIN improvements were 27/65 SPI-1005-treated (41.5%) vs 19/76 placebo-treated (25.0%), $p=0.0475$. In males ≥ 50 yo, HFPTA improvements were 7/31 SPI-1005-treated (22.6%) vs 2/40 placebo-treated (5.0%), $p=0.0357$. WIN improvements were 16/31 SPI-1005-treated (51.6%) vs 9/40 placebo-treated (22.5%), $p=0.0136$. In females ≥ 50 yo, PTA improvements were 7/34 SPI-1005-treated (20.6%) vs 2/36 placebo-treated (5.6%), $p=0.0802$. WIN improvements were 11/34 SPI-1005-treated (32.4%) vs 10/36 placebo-treated (27.8%), $p=0.7956$. During OLE, SPI-1005-treated subjects continued to show HFPTA (11-13%) and WIN (33-38%) improvements at 3- to 12-months.

Conclusions: MD patients treated with SPI-1005 demonstrated significant improvements in bilateral high-frequency hearing and speech discrimination characteristic of ARHL during RCT and OLE. These disease-modifying observations in an aged population > 50 yo support the future testing of SPI-1005 for the treatment of ARHL.

M14. Trajectory of Metabolic and Sensory Components of Age-Related Hearing Loss in 1.7 Million US Workers From the NIOSH Occupational Hearing Loss Surveillance Project

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Background: The current study investigated the longitudinal trajectory of age-related hearing loss (ARHL) across the adult lifespan and occupational noise effects. ARHL is theorized to

involve distinct cochlear pathologies that result from combined genetic and environmental risk factors with effects accumulated over the lifespan. Our theoretical framework is based on evidence that healthy outer hair cell function is critical for hearing sensitivity to low level sounds. Cochlear amplification can be reduced by a combination of outer hair cell losses (sensory) and declines in stria function that weaken outer hair cell motility (stria/metabolic). Sensory losses are associated with steep reductions in sensitivity to high-frequency sounds, while stria/metabolic declines affect sensitivity across lower- and higher-frequencies. Previously, self-reported noise exposure histories have provided support for noise-related sensory hearing losses. Our current goal was to further examine the role of occupational noise in ARHL with a population-scale, longitudinal audiogram collection that could be linked to noise exposure information.

Methods: Retrospective analyses were performed with a large audiogram dataset from US workers in 302 industry groups and 6 geographical regions (1.73 million participants, 77% men, 23% women; 18-75 years of age, average ~35 years; 1-6 audiograms collected at five-year intervals), originally collected as part of the NIOSH Occupational Hearing Loss Surveillance Project (1981-2010). Our previously developed model-fitting approach was used to estimate metabolic and sensory components of ARHL (in dB) per ear and audiogram, in addition to 4-frequency pure tone averages (0.5-4 kHz). Occupational noise measurements from the Job Exposure Matrix 2 were used to estimate noise levels for each worker's industry group. Regression-based analyses were then used to evaluate associations between metabolic or sensory estimates and each worker's baseline age, time across consecutive visits, sex, and noise exposure, while adjusting for US regions.

Results: Metabolic and sensory estimates both significantly increased with older age and across serial visits, with metabolic losses accelerating for older workers while sensory losses slowed. Men showed significantly higher sensory and metabolic estimates than women, and the sex differences significantly widened over time. Increasing occupational noise levels were significantly associated with higher sensory and metabolic estimates, and those noise exposure effects were significantly smaller for older than younger workers.

Conclusions: Our findings, based on up to 30 years of serial audiograms from nearly two million participants in the NIOSH dataset, expanded on earlier observations in the MUSC Longitudinal Cohort Study of ARHL and other cohorts. Consistent with previous observations, both components of ARHL increased with age, and men showed much higher sensory estimates than women. Interestingly, our results also suggest that noise exposure may worsen both components of ARHL, which reinforces the importance of hearing protection procedures and compliance with policies.

M15. Spectral Influences on Silent Gap Perception in the Aging Auditory Cortex

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Background: This study examined how older adults process silent gaps in auditory stimuli by recording cortical evoked potentials using a multi-deviant paradigm that is compared to a psychophysical gap-detection task.

Methods: Seventeen older adults (mean age = 63 years) with normal hearing or minimal hearing loss participated. Participants passively listened to pairs of noise markers separated by silent intervals. Markers were either spectrally identical (within-channel) or spectrally distinct (between-channel) and comprised both broadband and narrowband noise. Seven gap durations served as deviants in a multi-deviant sequence. The deviance-related negativity (DRN) and the P2/P3a complex were recorded from fronto-central electrodes.

Results: Broadband within-channel gaps significantly enhanced DRN amplitude. In narrowband conditions (within- and between-channel), only DRN was modulated; P2/P3a responses remained absent. Behavioral gap-detection thresholds did not differ across narrowband configurations, mirroring the ERP findings. For supra-threshold gaps, between-channel presentations produced reduced peak-to-peak amplitudes compared to broadband within-channel.

Conclusions: Older adults maintain robust neural sensitivity to silent gaps, reflected in DRN modulations that depend on the markers' spectral relationship. The absence of P2/P3a for spectrally narrow and distinct stimuli suggests limited pre-attentive engagement and may indicate age-related declines in temporal resolution or neural synchrony.

M16. Factors Influencing the Discontinuation of Cochlear Implants in Children With Autism Spectrum Disorder

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Background: To address significant hearing impairment, cochlear implants (CI) are often recommended as early as possible (Sharma et al., 2020), at an age when it is not always possible to identify other disorders (Cruz et al., 2012; Young et al., 2016). In recent years, the number of children with a CI who are later diagnosed with a co-occurring condition has increased (Edwards, 2007; Young et al., 2016); this includes children with autism spectrum disorder (ASD) (Edwards, 2007). There is considerable variability in CI outcomes among children with ASD (Robertson, 2013; Tavares et al., 2021), and 31% of children in this population use their CI only intermittently or not at all (Mathew et al., 2022). This study aims to identify factors that may explain the discontinuation or continued use of CIs among children with ASD. A better understanding of these factors could help clinicians optimize follow-up care for this population, especially given the current lack of research-based resources.

Methods: This retrospective and exploratory study includes children with or suspected of ASD (n = 11), aged 5 to 14 years, who received a CI as early as 2012. Participants' rehabilitation records were reviewed, and a telephone interview with a parent was conducted.

Results: Children were divided into two groups: the intermittent-use group (n = 6) and the consistent-use group (n = 5). The intermittent group included children who wore their CI for < 8 hours per day. On average, participants in this group wore their CI for 3.33 hours per day (SD = 2.94 h). Most had not acquired spoken language, had below-average cognition or suspected cognitive delays, and half exhibited behavioral issues. All had previously shown negative reactions to the CI, such as crying, refusal, or intentional damage. The consistent group included

children who wore their CI for at least 8 hours per day. On average, participants in this group wore their CI for 11.84 hours per day (SD = 2.06 h). Most had acquired spoken language, had average cognition, and no behavioral issues were noted.

Conclusions: This study provides a better understanding of CI use among autistic children and offers intervention strategies for clinicians, while also encouraging reflection on new clinical assessment techniques for children with neurodevelopmental disorders in future research.

M17. Delta-Band Cortical Speech Tracking Predicts Audiovisual Speech-In-Noise Benefit From Natural and Simplified Visual Cues

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Background: Visual speech cues improve speech-in-noise comprehension but it remains unclear which visual features are necessary and how they shape neural audiovisual (AV) integration. Prior work has tested both naturalistic faces and highly abstract envelope-driven visualisations, with the latter yielding mixed results. We aimed to determine how structural detail in visual signals modulates behavioural AV benefit and its neural correlates.

Methods: Two experiments were conducted. In a behavioural study (N = 19), participants performed a speech-in-noise comprehension task across conditions varying in visual detail: fully natural videos, edge-only faces, landmark-based cartoons, and abstract envelope-driven disks. In an EEG experiment, the same participants viewed longer audiovisual narratives from a single speaker, and neural tracking of the speech envelope was quantified using temporal response functions (TRFs) in the delta (1–4 Hz) and theta (4–8 Hz) bands.

Results: Behaviourally, AV benefit scaled with visual fidelity: natural faces produced the strongest improvements, edge and cartoon faces yielded weaker but reliable benefits, while disk stimuli produced none. Neurally, natural AV speech elicited a delta-band enhancement of auditory envelope tracking at ~180 ms, with similar trends for degraded faces. This delta-band AV–A difference correlated strongly with individual behavioural AV gains ($r = 0.52$, $p < 0.001$). By contrast, disk stimuli elicited no neural integration effects, instead producing SSVEP-like visual responses.

Conclusions: Facial structural detail is critical for AV speech benefit: even simplified faces provide measurable gains, whereas abstract envelope-driven signals do not. At the neural level, AV integration manifested not as a super-additive enhancement over summed uni-sensory responses, but as an increase in auditory envelope tracking and a reduction in visual envelope tracking. Overall, the results suggest a role of AV speech integration in enhancing auditory cortical encoding of word-rate dynamics. The findings could provide direction for the design of visual hearing aids or expressive virtual avatars.

M18. Hearing Aid Effectiveness and Probable Dementia Risk Across 33 Countries: A Pooled Analysis of Seven Cohorts

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Background: Hearing loss is recognized as a major modifiable risk factor for dementia, yet evidence linking hearing aid use to a lower risk of dementia is limited and inconsistent. This study aimed to evaluate whether the use of hearing aid—particularly effective use that improves hearing—is associated with a reduced risk of dementia in older adults with hearing loss and whether this association varies by level of economic development.

Methods: We used longitudinal data from seven international cohort studies (CHARLS, KLOSA, SHARE, ELSA, TILDA, HRS, MHAS). Hearing loss and hearing aid use were self-reported. Probable dementia was defined by cognitive impairment with functional decline or a physician diagnosis. We employed inverse probability of treatment weighting (IPTW)-corrected Cox proportional hazards models to address confounding. We estimated hazard ratios (HRs) for probable dementia by hearing aid use and hearing aid effectiveness in the harmonized cohort, and further conducted stratified analyses by country income level (High-income vs. Middle-income).

Results: The analysis included 61,089 hearing-impaired participants aged 55 and older from 33 countries. Over an average follow-up of 6.5 years, 8,911 participants (14.6%) developed probable dementia. In the IPTW-weighted analysis, hearing aid use was associated with a significantly lower risk of probable dementia risk (pooled HR 0.91, 95% CI 0.88-0.94). This association varied significantly, appearing stronger in settings with lower baseline hearing aid adoption, notably the middle-income countries (HR 0.76). Crucially, only participants who reported effective hearing improvement with their hearing aid showed a significant reduction in probable dementia risk (HR 0.86, 95% CI 0.80-0.93); those with poor hearing improvement had no significant benefit (HR 0.98, 95% CI 0.90-1.07). Subgroup analyses indicated a stronger association with a lower risk of probable dementia among participants aged ≥ 70 , women, those with lower education, lower family income, and unmarried individuals. Sensitivity analyses confirmed the robustness of findings.

Conclusions: Effective use of hearing aids was associated with substantially reduced probable dementia incidence, particularly among vulnerable populations. These findings highlight that

improving global access to high-quality hearing rehabilitation warrants consideration as a public health priority in efforts to address the global burden of dementia.

M19. The Pulse Polarity and Inter-Phase Gap Offset Effect in Patients With Low Frequency Residual Hearing After Cochlear Implantation

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Background: The auditory nerve (AN) status is important for the rehabilitation of patients with cochlear implant (CI), especially for patients with residual hearing (RH).

Methods: A total of 29 ears from patients with sensorineural hearing loss (SNHL) and LFPTA who underwent CI at our hospital were enrolled. All ears met the preoperative criteria, with hearing thresholds of ≤ 85 dB HL at 125–500 Hz, and > 85 dB HL at 1k–8 kHz. All ears were implanted with a CochlearTM Nucleus[®] device. The pulse polarity effect, including cathodic and anodic leading, as well as the inter-phase gap 35 (IPG35), were evaluated at electrodes 22, 12, and 3 after implantation. Parameters including slope of the input/output (I/O) function, maximum eCAP amplitude, eCAP threshold, and N1 latency were simultaneously analyzed.

Results: The mean acoustic hearings of all 29 ears were 55.0 dB, 65.7 dB, 91.1 dB, 102.3 dB, 105.7 dB, and 89.8 dB HL at 250, 500, 1K, 2K, 4K, and 8K Hz pre-CI. For cathodic results, the amplitude at C level of e22 was higher than e12, and e3 was higher than e12 ($p=0.0076$, $p=0.0378$); the threshold of e22 was lower than e12 and e3 was lower than e12 ($p < 0.0001$, $p=0.0005$); and the N1 latency at C level of e22 was shorter than e3 ($p=0.0454$). For e22, both the threshold and the N1 latency of cathodic were the same to anodic ($p < 0.05$). For e3, the threshold of cathodic was higher than anodic ($p=0.0039$), and the N1 latency for cathodic was longer than anodic ($p=0.0116$). The IPG35 offset compared to cathodic was the same for e22 and e3. For straight and pre-modiolar electrodes, the results differed in some parameters.

Conclusions: The apical electrode, which retains more residual hearing, exhibited a smaller pulse polarity effect compared to the basal electrode. This difference was particularly evident in the threshold and N1 latency parameters. Compared to IPG35, the pulse polarity proved to be more sensitive in distinguishing auditory neural function alongside different hearings.

M20. Extreme Neural Phase-Locking Across the Barn Owl's Lifespan

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Background: Barn owls are famous for the unique ability of their auditory-nerve fibres to represent temporal fine structure into the 10 kHz-range, which requires extremely low jitter in spike timing, of the order of 20 μ s. The mechanisms that enable this remain unknown, but likely involve specializations of the hair cells, their ribbon synapses with afferent neurones, and afferent axons. Here we report two series of experiments: first at various posthatching ages, documenting the emergence and maturation of auditory-nerve responses, and phase-locking in particular, and second, in aged barn owls, probing for age-related changes in temporal fine-structure representation.

Methods: Single-unit recordings were carried out with glass microelectrodes, under general anesthesia, either 1-1.5% isoflurane or ketamine/xylazine. Sound stimulation was through closed, individually calibrated earphones, situated at the entrance of the ipsilateral ear canal. Young barn owls were aged post-hatching day 11 to 130. Older individuals were classified either as adult (up to 4.5 years) or aged (8.8 – 11.8 years). Our definition of “aged” corresponds to about 50% of the species’ maximum lifespan potential and is approximately comparable to a 3-year-old gerbil, a 1.5-2-year-old mouse, or a 60-year-old human.

Results: Phase-locked responses were observed in all auditory-nerve fibres that were acoustically responsive, and from the earliest age tested. Following the typical avian developmental progression, only lower-frequency responses were found at the earliest ages, and progressively higher-frequency responses with advancing age. As in adult owls, phase-locking was typically observed from sound levels below rate threshold and vector strengths saturated at levels around 10dB above rate threshold. Maximal vector strengths were close to adult values from the earliest ages, but at high frequencies, > 5 -6 kHz, did not reach adult values until > 3 months of age. Phase-locking in aged barn owls was unchanged, i.e. no age-related decline was apparent. This was also true for additional metrics of fine-structure representation tested with broadband stimuli, derived from Wiener kernel and autocorrelogram analyses.

Conclusions: Compared to cat (Kettner et al., 1985, J Neurosci 5:275-283), maturation of neural phase locking in the barn owl is disproportionally prolonged in the specialized, highest-frequency regions of its hearing range. This is consistent with prolonged maturation also of CAP threshold sensitivity and amplitudes (Köppl and Nickel, 2007, J Comp Physiol A193:613-624), suggesting a generally delayed maturation of the very specialized, foveal regions of the barn owl cochlea. At the other end of the lifespan, our findings support the notion that birds have truly ageless ears; that is, they are able to preserve cochlear function to an advanced age – even in the barn owl, a species with extremely sensitive hearing and a specialized cochlea. It should be informative to probe the morphology of hair cells, ribbon synapses, and myelination across the lifespan for correlates.

M21. A Novel Mouse Model of Auditory Neurodegeneration via Ouabain Delivery Into the Posterior Semicircular Canal

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Background: Cochlear nerve damage has been reported to involve reactive oxygen species and glutamate; however, the detailed mechanisms remain unclear. In addition, because hair cell (HC) death can secondarily cause cochlear nerve damage, it is necessary to develop a selective model of cochlear nerve damage to elucidate the underlying mechanisms. Ouabain, a Na,K-ATPase inhibitor, has been reported to selectively eliminate spiral ganglion neurons (SGNs) when administered to the round window membrane (RWM) in mice (Yuan et al., J Assoc Res Otolaryngol. 2014). However, in practice, the strong pharmacological action of ouabain, the complexity of RWM administration, and individual variability among mice make it difficult to reliably establish a cochlear pathology model. Therefore, to enable more stable and selective inner ear delivery of ouabain, we investigated whether local ouabain administration to the posterior semicircular canal could generate a model of selective cochlear nerve injury.

Methods: The left retroauricular area of 8-week-old male CBA/J mice was incised. Under microscopic guidance, a small hole was created in the left posterior semicircular canal using a 32G needle. A micro-glass capillary was inserted into the opening, and either 1 mM or 5 mM ouabain or saline (control) was infused at 1 μ L/min using a microsyringe. Auditory brainstem response (ABR) was measured before and one week after administration, and threshold shifts and wave I amplitudes were compared between the groups. The inner ear was harvested one week after drug administration for histological evaluation.

Results: One week after 5 mM ouabain administration, a significant ABR threshold shift was observed compared to that in the controls ($p < 0.05$). Wave I amplitudes were reduced in the 1 mM and 5 mM ouabain-treated groups compared to those in the control group. A significant loss of SGNs was observed in both the apical and basal turns of the cochlea in the 5 mM ouabain group. In addition, 1 mM ouabain-treated groups showed a significant reduction in the number of cochlear synapses compared to the control group ($p < 0.05$). No loss of HCs was observed in any of the groups.

Conclusions: The results demonstrate that 5 mM ouabain administration induces significant threshold elevation and SGNs loss, successfully reproducing severe cochlear nerve damage. In contrast, although 1 mM ouabain did not cause marked SGN loss, it resulted in synaptic loss and a reduction in wave I amplitude, suggesting that it reproduces the pathophysiology of cochlear synaptopathy. This presentation also includes a literature-based discussion on the pathophysiological mechanisms of ouabain-induced auditory neurodegeneration via posterior semicircular canal administration.

M22. Intra-Operative Neural Health Measures: Relation to Etiology and Cochlear Implant Outcome

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Background: The electrically evoked compound action potential (eCAP) reflects the combined responsiveness to electrical stimulation of the spiral ganglion neurons (SGNs) that form the auditory nerve. In animal studies eCAP measures correlate strongly with numerical survival of SGNs, and with the health of the remaining SGNs. These correlations suggest that the eCAP is a potent predictor of hearing performance in human cochlear implant (CI) users, since it reflects the condition of the electrode-neural interface. However, correlations between eCAP measures and measures of hearing outcome are typically weak. Complicating factors in studies with human CI users include heterogeneity of the population (etiology, age, cognitive ability, duration of implantation), low patient numbers, and processing of clinically obtained data.

Methods: In the present study we examine the potential of the eCAP as a neural health measure in a population of > 150 MED-EL CI users. In this relatively large population the influence of etiology on eCAP measures can be studied. eCAPs were recorded with a short (2.1 μ s) and a long (30 μ s) inter-phase gap (IPG) using autoART in MED-EL's MAESTRO software. Both the absolute eCAP measures (e.g., amplitude, slope, dynamic range, latency) as well as their IPG effects were evaluated. Electrocochleography (ECoChG) was recorded in a subset (~40%) of patients.

Results: Preliminary results show moderate correlations between several eCAP measures and patient characteristics, such as age or duration of hearing loss. Etiology seemed to influence overall appearance of eCAP recordings.

Conclusions: Further analysis will reveal whether patient characteristics such as the extent of residual hearing, or etiology and duration of hearing loss play a significant role in the predictive value of the eCAP for CI performance.

M23. Potential Causes and Implications of Multi-Response Electrocochleography Electrode Sweep

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Background: During cochlear implant (CI) surgery, it is desirable to perform intraoperative, intracochlear recordings during electrode insertion to monitor the inner ear function to support the preservation of any residual hearing and to investigate cases of implantation-related residual hearing loss. One such method is intracochlear electrocochleography (ECoChG) which is the response of the inner and outer hair cells and the auditory nerve to acoustic stimuli. Although intracochlear ECoChG responses provide higher amplitudes as compared to round window recordings, significant challenges arise as the recording location of intracochlear ECoChG via the CI electrode changes during electrode insertion. Additionally, the acoustic stimulus is usually of a single frequency and not a compound of multiple frequencies where the subject might have residual hearing. This work aims to investigate the relationship between intracochlear ECoChG recordings during insertion and immediately after insertion, to understand the implications from patient audiogram and electrode location.

Methods: ECoChG responses were recorded in more than 35 CI subjects undergoing manual and robot-assisted CI surgery by two experienced surgeons in the same center. The acoustic stimulation to elicit this response was presented via Etymotic ER-3C insert earphones which were calibrated for Hearing Level (dB HL). The earphones were connected to an external acoustic stimulator which was programmed and triggered by the Maestro 9.0.4 based Research software (MED-EL GmbH, Innsbruck, Austria). The recordings were performed during and immediately after electrode insertion. During electrode insertion, alternating ECoChG measurements were made continuously as a response to a 12 ms long Chirp at 40 dB HL above the subject's pre-operative hearing threshold at 500 Hz. Immediately after the insertion, an electrode sweep was performed. Electrode sweep is a set of alternating ECoChG measurements from all 12 channels of the CI in response to the same acoustic stimuli. An 8 ms long burst at 500 Hz and at 40 dBHL above the subject's hearing threshold was used to perform the electrode sweep.

Results: We will present two categories of responses during insertion – first with multiple similar and large amplitude responses during insertion at different time points and second with only a single large amplitude response. Then the 500 Hz electrode sweep of these two categories will be compared. Finally, an estimate of the “final” tonotopic location of the channels along the electrode after full insertion will be made from CT images to investigate origins of responses in both categories.

Conclusions: This study hypothesizes that both thresholds and morphology of the preoperative audiogram influence the intracochlear ECochG. It is hypothesized to correlate with measurements made immediately after insertion, reflecting the tonotopic location during the electrode insertion, at least in interventions with residual hearing preservation.

M24. Temporal Processing Deficits in Cochlear Implant Users Assessed via Forward Masking With Direct Stimulation of Multiple Electrodes

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Background: As people age, one of the main contributors to poor speech perception is auditory temporal processing deficits. Although this has been extensively studied in acoustic listeners, the mechanisms underlying these deficits and how they occur in cochlear-implant (CI) users are not well understood. Furthermore, it is unknown whether these temporal processing deficits arise at the level of the inner hair cells and the auditory nerve (peripheral) or further up in the brain (central). CI users offer the advantage of studying these questions as the device completely bypasses the peripheral auditory system, aiding to understand differences in speech perception performance in CI listeners, especially as they age.

Methods: We recruited 12 CI users across the adult lifespan with Cochlear Ltd. devices. We evaluated their electrode-to-neural interface (ENI) by measuring electrically-evoked compound action potential amplitude growth functions (ECAP AGFs) to determine their “best” (steepest slope) and “worst” (shallowest slope) electrodes. We used the NIC4 system to generate an electrical broadband masker followed by a target electrical “chirp” while varying the masker-to-target interval (MTI) between 5-200 ms. We then stimulated multiple electrodes across the electrode array centered around their “best” electrode. While presenting the stimulus, we measured electrically evoked auditory brainstem responses (eABRs) and evaluated averaged eABR Wave V amplitudes and latencies. Separately, we performed a behavioral three-interval two-alternative forced-choice task using the same stimuli to evaluate detection thresholds for release from masking at each MTI.

Results: We initially hypothesized that with increasing age, the overall amplitudes of wave eV would decrease while latencies would increase, and that release from forward masking would be at an earlier time for younger than older CI users. In contrast, we found that increased duration of severe-to-profound hearing loss (DoSPL), and not age contributed significantly to decreased eV amplitudes and increased latencies. Furthermore, we found that with increasing MTI, the eV amplitudes increased and latencies decreased only in those participants with long DoSPL and poor ENI and did not significantly change in participants with shorter DoSPL and good ENI. These results demonstrate that central measures are correlated with peripheral physiological measures of ENI. Finally, these neural measures are correlated with behavioral release from forward masking regardless of age.

Conclusions: These findings suggest that ENI (peripheral) due to DoSPL at the time of implantation is the most important factor in the temporal processing deficits CI users may experience and that these are reflected downstream in the brain (central). By understanding the deficits at each neural locus, we can aim to treat these issues more effectively at the stages before

CI implantation as well as post-implantation through improving CI processing. Thus, the findings here can potentially influence future design of CI devices to better individualize treatment for patients in the clinic.

M25. Short-Term Improvements of Speech Perception Testing With Experimental Processing Strategies in Cochlear Implant Users

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Background: Capturing speech recognition abilities of cochlear implant users is difficult given the high variability of performance. A significant amount of this variability can be captured with peripheral estimates of the quality of the electrode-neuron interface. Estimates are based on detection thresholds obtained with focused electrical fields. To reduce negative effects of poor ENI quality, programming can be optimized for individuals using selective channel deactivation of high threshold channels and/or use of focused electrical fields. This study assessed three experimental maps that had high threshold channels deactivated and employed either monopolar, dynamic tripolar (DT) or a combination to remaining active channels, compared to subjects' clinical programs that served as a control. This study compared the difference in performance scores for IEEE sentences between the first (Run #1) and second (Run #2) sentence list in quiet and two conditions of background noise, an easy and hard condition. We hypothesized there would be a greater learning or improvements with experimental programs most different from the control, the conditions with focused electrical fields.

Methods: This study included 7 participants, aged 51-75 years (average age 64.7 years) with Advanced Bionics cochlear implants. One of the participants is bilaterally implanted and completed testing on both ears individually, for a total number of 8 ears tested. To identify high threshold channels for deactivation, participants first completed a fast sweep procedure to obtain thresholds across all channels using a highly focused electrical field (σ 0.9). Listener-tailored programs were then created using the Harmony research processor for each participant wherein high threshold channels were deactivated, and remaining channels were either MP, DT, or a combination of the two, mixed focused (MF). DT stimulation applies more focusing (σ 0.8) for low-intensity input sounds and less focusing (σ 0.5) for high-intensity sounds. A MF program uses DT on high threshold channels and MP for low threshold channels. For testing participants' clinical strategies, they were downloaded onto a Marvel and noise reduction was deactivated to best match processing in the Harmony. Speech perception testing was completed on a tablet in a sound booth across multiple sessions. Testing conditions were pseudo-randomized. Testing was completed in quiet and in noise with varying signal-to-noise ratios (SNRs) to assess a range of performance for easy and difficult conditions. For each SNR tested in each condition, two lists of 20 sentences each were administered. Audio responses were recorded on the tablet and scored later.

Results: Focused conditions—DT and MF—demonstrated the largest improvement in median percentages from Run 1 to Run 2, suggesting learning or acclimatization is occurring.

Conclusions: These preliminary findings suggest participants need more time with strategies involving focusing before they reach performance plateau and more than two runs may be necessary in future experiments.

M26. Auditory Spatiotemporal Interactions in Single-Sided Deaf Patients With Unilateral Cochlear Implants

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Background: There is growing use of unilateral cochlear implants (CIs) as a promising intervention for patients with single-sided deafness (SSD), especially to improve speech perception in noisy environments and sound localization performance. However, the effectiveness of CIs in improving spatial hearing for this population remains uncertain. Traditional sound localization tasks are often lengthy and cognitively taxing for individuals who struggle to identify sound source locations. This study introduces a new task for evaluating the restoration of auditory spatial cues in SSD-CI users by examining the biasing effects of spatial location on timing judgments for sequential sounds. The auditory spatial kappa (ASK) effect manifests as a bias in perceived relative onset timing between subsequent sounds as a function of the spatial distance between them. Sounds that are farther in space are judged as farther apart in time compared to sounds presented closer in space. We tested the degree of spatial bias on temporal judgments in SSD individuals with and without their CI as well as normal hearing controls to evaluate the restoration of spatial cues with CI in different frequency ranges. Crucially, participants are instructed to ignore the location of sounds, making this task a label-free measure of spatial cue restoration.

Methods: Participants heard sequences of three sounds played from loudspeakers, where the spatial distance between the first and third sound was fixed at 90 degrees apart (azimuth). The second sound changed location from trial-to-trial to be closer to the first or third sound. Participants made timing judgments by responding “short-long” to indicate that the second sound was closer in time to the first or “long-short” to indicate that the second sound was closer to the third. Stimuli were 200 ms narrowband noise bursts with low (500 Hz) or high (4kHz) center frequency to compare effects of predominant interaural timing or level cues, respectively. Sound sequences were presented within three sound fields: in front, on the right, or left side. Psychometric curves were fit to participant data to assess timing perception and response bias for each experimental condition.

Results: We found robust auditory spatial kappa (ASK) effects across all frequency and sound field conditions in normal hearing controls. Participants reported that the two sounds closer in space were closer in time. Preliminary results with SSD-CI participants show ASK effects for high but not low-frequency sounds without CI, indicating that monaural level cues for spatial location are sufficient to induce a temporal biasing effect. ASK effects were larger with CI in both frequencies, with effects moderated by sound field condition.

Conclusions: This study provides evidence that the novel ASK task may be a useful tool for implicitly assessing spatial hearing in clinical populations who perform poorly on traditional sound localization tasks.

M27. Improved Neural Adaptation by Integrating Voltage-Gated Ion Channels Into a Hybrid Phenomenological-Biophysical Model of Electrical Stimulation of the Auditory Nerve

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Background: Computational models of electrical stimulation of auditory nerve fibers (ANFs) contribute to the understanding of physiological and perceptual phenomena of electric hearing, thereby helping to improve the development of novel cochlear implant coding strategies. The hybrid phenomenological-biophysical model of Joshi et al. (2017) aims to balance computational simplicity with sufficient physiological detail and achieves accurate predictions of a wide range of physiological phenomena. However, the model also demonstrates weaknesses in terms of limited neural adaptation for high-rate stimulation pulse trains and prediction of abnormally high spontaneous firing in ANFs. Additionally, its ability to explain the dependence of the dynamic range of firing efficiency (i.e., relative spread) on the pulse phase durations (PPDs) is an artifact of how the model code is initialized. An improved parameterization of the model (Bruce, ARO 2024) reduced the dependence on pulse onset timing and the spontaneous firing behavior, however leading to a loss of the monotonically growing relative spread (RS) vs. PPD relationship observed in physiological data. This is likely caused by a missing mechanism in the model accounting for the RS vs. PPD relationship and spike-rate adaptation.

Methods: In this study we replaced the phenomenological supra- and subthreshold adaptation mechanisms in the Joshi et al. model by voltage-gated KLT and HCN ion channels from a biophysical model (Negm and Bruce, 2014), which have shown to predict a strong adaptation behavior in ANFs for high-rate pulse trains and can explain the growing RS vs. PPD relationship (Negm and Bruce, 2008). After calibrating the adaptation currents of the voltage-gated ion channels with respect to the membrane noise sources and the exponential integrate-and-fire mechanism of the Joshi et al. model, the RS was measured as a function of the PPD for different pulse onset times. In addition, the adaptation behavior was quantified for low- and high-rate pulse trains via post-stimulus time histograms.

Results: The simulation results demonstrate a considerably reduced dependence of pulse onset times on the RS while predicting the monotonically growing RS vs. PPD relationship observed in physiological measurements. Moreover, after including the voltage-gated ion channels in the model a strong spike-rate adaptation behavior can be observed for high-rate stimulation pulse trains.

Conclusions: Replacing the phenomenological supra- and subthreshold mechanisms of the Joshi et al. model by biophysically inspired voltage-gated ion channels improves the spike-rate adaptation behavior of the model, reduces the dependence of the pulse onset time on the RS, and predicts the growing RS vs. PPD relationship observed in physiological data. Additional refinements such as modeling a more biophysically realistic spike waveform may yield further improvements in terms of the adaptation behavior.

M28. Cochlear Microphonic, Compound Action Potential and Pure Tone Audiometry in the First Year After Cochlear Implantation

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Background: Electrocochleography (ECoChG) has become increasingly valuable in cochlear implant (CI) surgery as it allows intraoperative monitoring of effects of electrode array insertion on residual hair cell function. Additionally, ECoChG has the potential to portray postoperative cochlear function. Here we examine the postoperative time course of residual hair cell function by recording ECoChG in CI recipients intraoperatively and at several time points up to one year postoperatively.

Methods: Twenty-four patients with severe sensorineural hearing loss receiving a CI participated in a trial comparing electrode arrays (SlimJ and Mid-Scala of Advanced Bionics) and surgical approaches (Jwair et al., Trials, 2021). Here, the data was analyzed by investigators blinded to the randomization. ECoChG recordings were performed at 5 time points using the active insertion monitoring (AIM) system of Advanced Bionics: intraoperatively, at 6 weeks, 3 months, 6 months and 12 months postoperatively. Intraoperative recordings and recordings at 6 weeks and 3 months postoperative were obtained from 23/24 participants. Postoperative recordings at 6 months were obtained from all 24 participants. Postoperative recordings at 12 months were obtained from 22/24 participants. Responses to pure-tone stimuli with frequencies varying from 125 to 4000 Hz, at sound levels 100-115 dB HL, were recorded at each of the 16 available electrodes, in two opposite phases. The difference and sum of the recordings to the opposite phases were computed as estimates of cochlear microphonics (CM, reflecting hair cell potentials) and compound action potentials (CAP, reflecting auditory nerve responses), respectively. Mean CM and CAP amplitudes of responses will be compared to hearing thresholds from pure tone audiometry.

Results: Significant CM responses were found in 14 out of 23 participants intraoperatively, and postoperatively in 21/23 participants at 6 weeks, 22/23 at 3 months, 23/24 at 6 months, and 23/23 at 12 months. Substantial CM responses ($> 1.5 \mu\text{V}$) were found in 13 out of 23 participants intraoperatively, and postoperatively in 3/23 participants at 6 weeks, 3/23 at 3 months, 5/24 at 6 months, and 4/23 at 12 months. Notably, 3 intraoperative non-responders showed significant responses in the first postoperative session. Averaged across frequencies 125, 250 and 500 Hz, the largest responses were observed recorded at the apical electrodes 1 – 5 during the intraoperative session. The responses were smallest at 4-6 weeks postoperatively, then recovered after 3 to 7 months, and decreased again up to one year after implantation.

Conclusions: Residual hair cell function gradually declines over the course of one year. Three months after cochlear implantation a short period of recovery is seen, which may be attributed to natural reduction of the acute local inflammatory response to the electrode array.

M29. Co-Registered 3D X-Ray Microscopy and Cubic-Shield Cleared Whole-Cochlea Imaging Demonstrate Co-Localization of Post-Implantation Neo-Angiogenesis and Neo-Ossification Foci in Mice

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Background: Cochlear implants (CIs) are a well-established neuroprosthetic intervention for hearing loss, yet outcomes remain variable, particularly in speech perception and hearing preservation. This variability is multifactorial, but cochlear neo-ossification, part of the foreign body response to implantation, has been linked to poorer neural health and speech understanding. The relatively hypoxic milieu of the scala tympani suggests a role for hypoxia-driven angiogenesis in post-CI neo-ossification. We employed complementary 3D X-ray Microscopy (3DXRM) and whole-cochlea CUBIC-SHIELD clearing to examine temporospatial relationships between post-CI angiogenesis and ossification.

Methods: Ten- to twelve-week-old PECAM+/GFP C57Bl6 mice with normal hearing underwent unilateral cochlear implantation. Cochleae were harvested at 10 (n=6) or 28 (n=6) days post-implantation and fixed. Mineralized bone was detected via 3DXRM, while PECAM+/GFP reporters labeled new vasculature. Whole cochleae underwent 3DXRM prior to CUBIC-SHIELD clearing, DAPI counterstaining, and immunolabeling with anti-GFP and anti- α -SMA. Cleared specimens were imaged by confocal microscopy, and reconstructed z-stacks were co-registered with 3DXRM datasets to assess ossification and angiogenesis adjacent to the implant tract.

Results: CUBIC-SHIELD clearing preserved PECAM-GFP reporter signals and revealed marked vascular proliferation and α -SMA-positive fibrosis in the basal scala tympani encircling the implant, with prominent vascular arcades near the round window. Anti-GFP staining corroborated reporter expression. At 10 days post-implantation, preliminary analyses demonstrated spatial co-localization of early ossification foci with areas of angiogenesis, particularly in the basal turn adjacent to the round window.

Conclusions: CUBIC-SHIELD clearing enables whole-cochlea imaging with preservation of reporter signals and compatibility with immunolabeling. Integration of 3DXRM and CUBIC-SHIELD yields complementary datasets that can be co-registered to characterize both mineralized (neo-ossification) and soft-tissue (fibrosis, angiogenesis) components of the post-CI foreign body response. These findings suggest that robust neo-angiogenesis may support inflammatory cell infiltration and foreign body reactions within the normally hypoxic, acellular scala tympani, however further studies are needed to investigate this hypothesis. Angiogenesis may represent a modifiable therapeutic target to mitigate deleterious post-CI tissue responses.

M30. Outcomes for Revision Cochlear Implant Surgery - Perimodiolar to Straight Electrodes

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Background: It is common practice to replace perimodiolar electrodes with straight electrodes during revision cochlear implant (CI) surgery. The electrode spacing and contact positions differ between these electrode types. In particular, the most basal contact of the Slim Straight electrode sits approximately 4 mm deeper than the Slim Perimodiolar or Contour Advance electrodes, which may alter pitch perception. This type of revision surgery may also result in different modiolar proximity due to electrode location and fibrous tissue growth around the

original electrode, potentially further altering pitch perception and hearing outcomes. This study aims to investigate how reimplantation with the Slim Straight electrode after original implantation with the Slim Perimodiolar or Contour Advance electrodes affects patient outcomes, basal contact modiolar proximity, and stimulation levels.

Methods: This was a single-institution retrospective cohort study of patients who received revision CI with a Slim Straight electrode following implantation with a Slim Perimodiolar or Contour Advance electrode from 2020-2025. Patients were included if they underwent revision surgery at our institution for any cause. Etiology of hearing loss, cochlear anatomy, surgery dates, reasons for revision, and documentation on sound quality were abstracted from patient records. Speech perception scores, X-rays, and mapping parameters, including frequency allocation tables (FATs) and lower and upper stimulation levels before and after revision, were analyzed.

Results: 17 implanted ears from 16 patients were included in this study. Ages at time of revision ranged from 1.3 to 86.4 years (mean 20.1, SD 30.7). All included patients had normal ear anatomy. Reasons for revision included device infection (76.5%), failure (17.6%), and migration (5.9%). The high frequency edge of the filter associated with the most basal electrode was 7938 Hz for all patients before and after revision. The low frequency edge of the filter was typically 6938 Hz, with two exceptions (Patient X, pre-revision, 7793 Hz; Patient Y, post-revision, 6563 Hz). Comparison of threshold levels demonstrated significantly higher thresholds in the most basal electrode before versus after revision (140.5 vs 121.3, $p < 0.05$). Speech perception was evaluated in 5 patients, with no difference in mean scores before versus after revision (CNC: pre-revision 47.8%; post-revision 43.4%, $p=0.79$. AZ-Bio: pre-revision 42%; post revision 59.4%, $p=0.39$). Review of qualitative data demonstrated lack of documentation on pitch perception. X-ray data will be further explored.

Conclusions: Speech perception data suggests reimplantation with an electrode of different design and spacing does not alter frequency allocation or overall speech perception. We anticipate analysis of X-rays to confirm deeper location of the Slim Straight electrode and explore whether straight electrodes adopt modiolar proximity due to fibrous tracts from prior implantation with perimodiolar electrodes. A prospective study evaluating pitch perception among this population is warranted to assess impacts of changing electrode contact location on overall hearing quality.

M31. DeepFS4: An End-To-End Deep Learning Based Cochlear Implant Sound Coding Strategy

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Background: Cochlear implant (CI) users typically achieve good speech understanding in quiet environments. However, intelligibility declines significantly in the presence of background noise. Conventional front-end speech enhancement techniques can mitigate this effect, yet often introduce latency, work primarily with stationary noise, and do not fully exploit the CI sound coding chain. Recently, DeepACE, an end-to-end neural network fully replacing the Advanced Combination Encoder (ACE), demonstrated that tightly integrated, learnable CI strategies can improve intelligibility without added latency. However, no such approach has yet been

developed for sound coding strategies that encode temporal fine structure (FS), such as the FS4 strategy, which determines low-frequency channel pulse timings from zero-crossings in the audio signal to represent neural phase locking in the auditory nerve.

Methods: We developed DeepFS4, a temporal convolutional network designed to replicate FS4 outputs while simultaneously embedding speech enhancement. The architecture is compact, with fewer parameters than comparable models, making it feasible for real-time, low-power CI devices. To assess the impact of FS encoding on speech intelligibility, two variants were tested, one with ideal FS locations and the other with noisy ones. Objective evaluations included the signal-to-noise ratio improvement (SNRi), while the speech intelligibility was evaluated with ten CI recipients in stationary and non-stationary noise. These tests consisted of the German HSM sentence test (Word Recognition Score), a MUSHRA quality rating, and a 3-AFC test to examine FS location effects.

Results: DeepFS4 achieved a mean SNRi of 8 dB across input SNRs from −5 to 10 dB for stationary and 6 dB SNRi for non-stationary noise. In listening tests, it yielded significantly higher word recognition scores and higher subjective quality ratings than both the commercial FS4 strategy and state-of-the-art front-end enhancement algorithms. The comparison between the DeepFS4 variants of the FS encoding revealed negligible differences in speech intelligibility and user detectability.

Conclusions: DeepFS4 demonstrates that integrating speech enhancement directly into the sound coding strategy via a low-latency deep learning architecture can substantially improve CI speech perception in noise. The results demonstrate that robust performance improvements are possible even without FS location enhancements, encouraging simpler and more streamlined designs in future research. The approach introduces a pathway towards the first AI-powered, clinically viable sound coding strategy for CIs, with direct relevance for improving real-world listening outcomes.

M32. Contralateral Sound Attenuation Can Help Hearing-Aid Users Understand Speech in Realistic, Dynamic “Cocktail Party” Listening Situations

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Background: Hearing aids (HAs) can restore audibility and loudness perception, but users often complain that they struggle to understand speech in noisy, “cocktail-party”-like listening situations. We have shown elsewhere that contralateral sound attenuation (CSA) (Lopez-Poveda et al., 2022, Hear Res 409:108469) can help HA users in these situations by improving the signal-to-noise ratio in the acoustically better ear (San-Victoriano et al., 2023, Hear Res 432:108743). The evidence, however, was restricted to free-field simulations where the better ear was known to the listener and fixed across trials, which is far from realistic. Here, the aim was to assess the potential benefits of CSA in more realistic conditions with reverberation and changing the masker location dynamically from trial to trial to make the better ear uncertain to the listener.

Methods: 15 users of bilateral HAs were asked to recognize the key words of sentences presented in competition with five simultaneous masking sounds. Stimuli were delivered via loudspeakers mounted on a ring located in a room with low reverberation ($t_{60}=20$ ms). The target (a female speaker, 65 dB SPL) was in front of the listener (0-degree azimuth). Four of the maskers (international female fluctuating maskers, 60 dB SPL) were symmetrically placed around the listener (at ± 45 -degree and ± 135 -degree azimuth). The fifth masker was speech-shaped noise (SSN) and its level was previously determined to avoid ceiling and floor effects on recognition. Importantly, the location of the SSN source varied randomly within the arc between ± 120 -degree for each sentence presentation. Participants performed the task while listening with the left ear alone, the right ear alone, or the two ears. To make this possible with life sounds, stimuli at either or both ears were picked up with an acoustic mannikin placed in the center of ring and sent to the listener, who was seated in a separate room. Sounds were either unprocessed or processed through the CSA algorithm before they were processed through a software HA that applied individualized nonlinear, frequency-specific amplification.

Results: CSA significantly improved speech recognition in bilateral listening [$F(1,14) = 19.0$, $p < 0.001$], and in unilateral listening with the left ear [$F(1,14) = 40.4$, $p < 0.001$] or the right ear [$F(1,14) = 67.5$, $p < 0.001$]. The benefit of CSA was significantly greater for SSN source locations further away from the target

Conclusions: Contralateral sound attenuation can improve speech intelligibility for HA users in realistic (reverberant and dynamic) multitalker listening situations. (Work supported by a predoctoral contract of Junta de Castilla y León to PGZ, and by MED-EL GmbH).

M33. Experimental Programming for Cochlear Implants: Dynamically Focused Stimulation Combined With Monopolar Edge Electrodes

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Background: Channel interaction, or activation of overlapping neural populations, is one likely contributing factor to cochlear implant (CI) users' difficulties in understanding speech in noise and music. Clinical strategies in CIs use monopolar (MP) stimulation, which results in broad current spread that contributes to channel interaction. Current focusing with adjacent electrodes is one potential solution to reduce spread. Experimental programs using current focusing to reduce channel interaction have shown potential benefit; however, these programs have only been tested using 14 of 16 electrodes, as the electrodes at the edge of the array have no flanking electrodes to use for focusing and are therefore deactivated. Using these edge electrodes with monopolar stimulation may provide better spectral resolution by reducing channel filter size and fuller sound quality by stimulating spiral ganglion neurons at the edges of the electrode array that were previously un-stimulated in focused programs. In this study, participants were tested on speech in noise and timbre instrument recognition using three programs: a 14-channel monopolar program (MP14), a 14-channel dynamic tripolar program (DT14), and a 16-channel dynamic tripolar program with electrodes 1 and 16 active as monopolar electrodes (DT16).

Methods: At this point, data have been collected from 11 ears in 9 adult participants (mean age = 50.9 years, SD = 19.4 years). All participants used Advanced Bionics devices and were tested across two sessions. In session one, three experimental programs were created: auditory

detection thresholds and max comfort levels were first measured across all electrodes to be used as the programming levels, which were further fine-tuned using live speech. Participants finished session one by completing the test battery using their clinical device as training. The test battery consisted of the instrument timbre recognition task from the UW CAMP test, closed-set /hVd/ vowel identification in noise, and the closed-set American English Matrix Test (AMT) in adaptive noise. In session two, participants completed the test battery for each program, with program order block-randomized across participants. Data was analyzed using linear-mixed effects models to determine the effect of program.

Results: Planned comparisons showed no significant mean difference between MP14 and DT14 (testing the effect of focusing) and DT14 and DT16 (testing the effect of MP edge electrodes), with high variability across subjects. Despite incorporating additional training during session one and increased practice trials for each program during session two, the timbre task shows significant learning with test order ($\beta = 5.81\%$ /test block, $p = .028$). AMT and vowels were stable across test order.

Conclusions: Reincorporating electrodes 1 and 16 as monopolar electrodes in a dynamically focusing program show no clear benefit in this interim analysis, but data collection is still underway. It is possible that the added interaction from these electrodes contributed to this lack of benefit.

M34. Musical Context Influences the Impact of Vocal Boost Manipulations for Cochlear Implant Users

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Background: Music perception and enjoyment remain a persistent challenge for post-lingually deafened adults with cochlear implants (CIs), largely due to the degraded spectral cues essential for melodic processing. Vocal boosting - enhancing the levels of vocals relative to background - has been proposed as a means of improving sound quality and enjoyment in this population. Yet, whether the benefit of vocal boosting derives from greater access to lyrical content within a coherent musical scene, as opposed to increased audibility over a non-musical masker, remains unclear. This study investigates how the perceptual gains from vocal boosting depend on the acoustic nature of the background.

Methods: Fourteen CI users rated the sound quality of stimuli on a 100-point scale. Vocals from ten popular songs were extracted and paired with three types of backgrounds: original instrumentals, off-key instrumentals (+4 semitones), and multi-talker babble. Each vocal-background pair was normalized to have an equal signal-to-noise ratio (SNR), forming the +0 dB SNR condition. Stimuli were then manipulated to create +9 dB (boosted) and -9 dB (attenuated) SNR conditions. An anchor stimulus (1,000 Hz low-pass filter with white noise) was included. Participants rated stimuli relative to the +0 dB SNR condition for each background. A subsequent forced-choice test assessed recognition of the original song from the three background types.

Results: Background type had a dominant impact on sound quality ratings ($\eta^2=0.68$), exceeding the vocal level effect ($\eta^2=0.35$). Vocals over both original and off-key instrumental backgrounds were rated similarly (58.2 vs. 56.9; $p=0.774$) and significantly higher than vocals over babble noise (22.9-point difference, $p < 0.001$). Thus, CI users reliably differentiated musical from non-musical backgrounds, but were insensitive to large pitch distortions. Vocal boost (+9 dB) increased ratings modestly (+ 7.2 points; $p < 0.05$), while attenuation (-9 dB) produced a more pronounced decline (-14.7 points; $p < 0.001$). Results revealed a significant interaction between background type and vocal level ($F(2,39)=11.31$, $p < 0.01$), with vocal boost effects varying by background type: babble showed the largest improvement (+16.6 points, $p < 0.001$), followed by original instrumental (+7.2 points, $p=0.001$) and off-key instrumental (+4.1 points, $p=0.122$). Overall song recognition accuracy was 68%. Participants with a history of musical training showed a significant advantage in recognition (75% vs. 61%, Cohen's $d=0.31$) and derived greater benefit from vocal manipulations.

Conclusions: While vocal boosting provides benefit across all background types, the highest absolute sound quality ratings emerge when vocals are embedded within a coherent musical structure, highlighting the importance of preserving contextual cues rather than simply amplifying speech. The ability to distinguish musical from non-musical content, coupled with insensitivity to pitch inaccuracy, underscores a reliance on structural over spectral fidelity. These findings suggest that future CI musical processing strategies should retain musical scaffolding, while selectively boosting vocal prominence to meaningfully improve musical enjoyment.

M35. Impact of Percussive Rhythmic Cues With Different Timbres on Music Sound Quality Ratings in Cochlear Implant Users

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Background: Music perception is a significant challenge for cochlear implant (CI) users due to device limitations that degrade the spectral cues necessary for pitch and timbre perception. In contrast, the perception of rhythm, which is conveyed by temporal envelope cues, is largely preserved. Consequently, CI users often rely on temporal and rhythmic information to parse complex auditory scenes. However, it remains unknown whether the deliberate enhancement of music with salient rhythmic textures can improve subjective sound quality. This study investigated how CI users' and normal hearing (NH) listeners' ratings of musical sound quality are affected by overlaying piano music with percussive textures that differ in timbre and frequency ranges.

Methods: An online sound quality rating task was completed by 13 experienced CI users and 14 NH controls. Participants rated ten instrumental piano excerpts under six conditions: an unaltered Reference, a degraded Anchor (1,000 Hz low-pass filter with noise), and four conditions where a distinct percussive texture was overlaid. The textures, equated for rhythmic complexity and RMS-normalized, were: a low-frequency Membrane (300-500 Hz), a mid-frequency Woods (500-4,000 Hz), a high-frequency Metallics (4,000-8,500 Hz), and a full-spectrum Mixed (300-8,500 Hz). A mixed-effects ANOVA was used to analyze differences in ratings between groups and conditions.

Results: A significant interaction between condition and hearing group was found ($F(5,1595)=31.2$, $p < 0.001$, $\eta^2=0.089$), revealing opposing patterns of preference. Compared to the Reference condition, CI users provided significantly higher ratings for the mid-frequency Woods ($p < 0.001$), higher-frequency Metallics ($p=0.012$), and full-spectrum Mixed ($p=0.045$) overlays. Conversely, NH listeners rated the mid-frequency Woods ($p < 0.001$) and full-spectrum Mixed ($p=0.003$) overlays significantly worse than the Reference. For the CI group, the largest enhancement was observed for the Woods condition (+27.3 points; Cohen's $d=1.35$). No significant group differences were found for the Reference ($p=0.76$), Anchor ($p=0.69$), or Membrane ($p=0.80$) conditions.

Conclusions: Cochlear implant users experience a significant enhancement in musical sound quality when complex piano music is augmented with salient, rhythmically congruent percussive textures. This effect, which is opposite to that observed in NH listeners, is most pronounced for textures with substantial mid-frequency energy. These findings suggest that leveraging CI users' preserved temporal processing abilities via textural enhancement may be a useful tool to improve musical enjoyment. This work has implications for the development of front-end, user-centric music mixing strategies for CI music perception.

M36. A Machine Learning Framework for Predicting Vowel Confusion in Cochlear Implant Users

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Background: Vowel perception, in part, reflects the auditory system's ability to extract and utilize spectral peaks (formant frequencies). Therefore, identifying vowel confusion patterns may highlight frequency regions where formant cues are degraded and guide targeted cochlear implant (CI) programming. Current approaches rely on time-intensive, reactive testing to identify problematic vowel contrasts. This study aimed to develop and validate a machine learning framework that predicts vowel confusion patterns from clinical and psychophysical data, enabling rapid, clinically feasible, data-driven insights into the electrode-neural interface (ENI).

Methods: We analyzed 1,319 vowel identification trials from six adult CI users tested in noise with a 9-vowel corpus (/u/, /ʊ/, /oʊ/, /ɑ/, /ʌ/, /æ/, /ɛ/, /ɪ/, /i/) presented in a nonsense syllable context. Multi-modal data were flattened through feature engineering, aggregating electrode measurements and psychophysical data into subject-level summary statistics while converting temporal sequences and acoustic properties into scalar features. Input features were multi-modal, including: (1) acoustic parameters (formant frequencies, electrode-frequency mapping); (2) Psychophysical measures of the ENI, such as focused stimulation thresholds and psychophysical tuning curve slopes; and (3) performance metrics (historical confusion matrices, within-session trial effects). The predictive framework combined five heterogeneous base learners (random forest, gradient boosting, neural networks, support vector machines, k-nearest neighbors) in an ensemble model. Feature selection was optimized using mutual information analysis to capture non-linear dependencies. Model performance was validated with leave-one-subject-out cross-validation and assessed for stability via bootstrap resampling. The primary outcome was prediction accuracy for the 9-class vowel classification task.

Results: The ensemble model achieved 92.4% cross-validated accuracy (95% CI: 91.0%–93.8%) and a macro F1-score of 0.924 in predicting the specific vowel response on a per-trial basis. Model performance was strongly dependent on ENI integrity, with accuracy ranging from 96%–99% for subjects with robust ENI to 48% for those with poor ENI. The model demonstrated robust performance across cochlear regions (89.5%–94.8%) while identifying clinically relevant confusion patterns, including systematic front vowel substitutions (/eɪ/→/ɛ/: 9.8%). Feature ranking via mutual information analysis confirmed that ENI measures (normalized MI: 0.41) and historical confusion matrix (0.35) were the strongest predictors, outweighing the vowel's acoustic formant structure (0.18). This ranking establishes that individual neural response characteristics exert greater influence on vowel perception than acoustic parameters alone.

Conclusions: This study introduces a validated machine learning framework capable of predicting specific vowel confusion patterns in CI users. These preliminary results establish that the fidelity of ENI as an important determinant of vowel perception outcomes. Potential applications include leveraging vowel identification tasks to infer ENI integrity without relying on time-intensive psychophysical testing. Future translation will require integration into existing audiology workflows, and prospective validation in larger, more diverse patient cohorts.

M37. Open Board

M38. The Role of Electrode-Neural Interface Viability on Timbre Perception and Reorganization Following Electrode Deactivation in Cochlear Implant Users

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Background: Music perception remains a major challenge for cochlear implant (CI) users. While the integrity of the electrode-neural interface (ENI) has a known effect on speech-in-noise outcomes, its role in perceiving musical features such as timbre is less clear. This study aimed to (1) identify psychophysical correlates of timbre perception and (2) assess whether a targeted programming intervention—deactivating electrodes with high stimulation thresholds—modifies timbre performance.

Methods: Thirteen adult CI users participated. Forward and reverse sweep-focused thresholds were obtained for electrodes 2 through 15. The stimulus levels were averaged across all trials. ENI uniformity, a measure of the consistency of the interface between the electrodes and auditory neurons, was assessed using the standard deviation of the differences in thresholds between neighboring electrodes. Frequency selectivity, which reflects the sharpness of neural activation patterns, was measured using the slopes of psychophysical tuning curves (PTCs). In the first phase, we performed a Pearson correlation analysis to relate ENI uniformity and PTC slopes to timbre identification on the CAMP test. In the second, interventional phase, we compared user performance on the timbre task with their everyday clinical program and an

experimental program where two to five electrodes with the highest stimulation thresholds were acutely deactivated. Changes in accuracy and perceptual confusion patterns were analyzed using paired t-tests and Chi-square analysis, respectively.

Results: PTC slope, rather than ENI uniformity, was a significant predictor of timbre perception. The PTC slope at high-threshold electrodes was a strong negative predictor of overall timbre perception ($r=-0.66$) and specifically for the strings/percussion category ($r=-0.70$). In the interventional phase, deactivating high-threshold electrodes did not significantly alter overall timbre identification accuracy (42.6% vs 43.9%). However, this stable accuracy masked a profound underlying perceptual reorganization, confirmed by a shift in the pattern of instrument confusion errors ($\chi^2(112)=698.19$, $p < 0.001$). The total perceptual distance between the programs was 16.1%, indicating a moderate but meaningful change in error patterns. This reorganization was systematically linked to acoustic features; instruments with a low spectral centroid showed moderate stability (15.8% distance), whereas those with a medium spectral centroid were the most affected (17.3% distance). Among the eight instruments, piano showed the greatest stability (12.8% distance), while guitar demonstrated the largest reorganization (20.5% distance).

Conclusions: The sharpness of PTCs is a key predictor of CI-mediated timbre perception. Targeted electrode deactivation acutely modifies spectral cue processing without altering global accuracy, demonstrating neural plasticity and reweighting of acoustic information. The paradoxical improvement in identifying some instruments alongside the decline of others highlights the importance of analyzing perceptual confusions, not just accuracy scores, to understand how CI users adapt to their signal. Future tonotopic analyses are needed to link these perceptual shifts to the spectral properties of the deactivated electrodes.

M39. Patient Preferences for Hearing Aid Features and Service Delivery Models

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Background: Patient preferences play a critical role in hearing aid adoption and sustained use. As new hearing device features evolve and new service delivery pathways emerge, including over-the-counter (OTC) devices, it is increasingly important to understand how adults with hearing loss value device features and professional services. Financial considerations and convenience may draw some patients toward OTC options, yet many individuals may continue to prioritize advanced device features and professional involvement in their care. The current study explored the patient preferences for hearing aid features and service delivery models.

Methods: This study is nested in a four-arm randomized clinical trial and used a cross-sectional survey design. A total of 114 adults with self-reported hearing disability completed the participant profile questionnaire. The questionnaire collected demographic information along with preferences for hearing device features and service delivery models. Responses were analyzed descriptively.

Results: The average age of participants was 66 years; 73% were female. Most held at least a university degree (77%), and more than half were retired (53%), with 28% employed full time. Thirty-nine percent reported annual household incomes between \$50,000 and \$99,999, and 28% lived alone. Regarding device features, participants rated the ability to hear friends and family in noisy environments, physical comfort, and device reliability as highly important. For service delivery, prescription hearing aids with in-person support from a hearing healthcare professional were rated most favorably, whereas OTC devices with online technician support were least preferred. Cost was considered an important factor, but the role of healthcare professionals in the decision-making process was rated as more important. Most participants reported feeling moderately to extremely uncomfortable with purchasing a hearing aid without a prior hearing test.

Conclusions: Adults with self-reported hearing disability placed a high value on interpersonal communication in noise, comfort, and reliability when considering hearing aid features. The strong preference for professional guidance highlights the continued importance of hearing healthcare professionals in facilitating hearing aid journey. This highlights the importance of professional support despite the availability of OTC devices and virtual models.

M40. Relation Between Financial Toxicity and Hearing Aid Preferences

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Background: Financial toxicity, defined as the financial distress or hardship associated with healthcare costs, may influence decision-making among individuals with hearing loss. Patients experiencing higher financial burden are likely to consider low-cost hearing devices, even if they express preferences for professionally fitted devices or advanced features. Understanding how financial strain shapes hearing aid feature and service delivery preferences can provide valuable insights for tailoring patient-centered care and informing policy decisions. The purpose of this study is to examine the relationship between financial toxicity, demographic and audiologic factors, and preferences for hearing aid features and service delivery models among adults with self-reported hearing disability.

Methods: This study is nested in a four-arm randomized clinical trial and used a cross-sectional survey design. Data were collected from 114 adults with self-reported hearing disability who completed a patient profile and financial toxicity questionnaires. The questionnaire assessed demographics, hearing aid features, and preferences for service delivery models. Associations between financial toxicity, demographic variables, and preferences for hearing aids were examined using Spearman correlation coefficients. For preferences with nominal responses (> 2 categories), standardized mean differences were calculated to evaluate differences in demographic variables and financial toxicity across preferences.

Results: Eighty-two (76%) had Comprehensive Score for Financial Toxicity (COST) Grade 0 (No impact, score $\geq$ 26) for financial toxicity. Higher financial toxicity was moderately correlated with having Medicaid insurance and with cost being an important factor in choosing hearing aid service delivery models. Participants who rated affordability as the most important factor

influencing their decision to purchase a over-the-counter (OTC) hearing aids were more likely to be working at least part time and younger than those who prioritized initial fitting by a healthcare professional or ability to adjust the sound (medium-to-large effect). Preference for advanced OTC hearing aid features was more common among individuals with six-figure incomes compared to those preferring moderate features (large effect). Participants with a stronger preference for online support and assistance with OTC devices compared to in-person support were more likely to be Medicaid-insured (large effect). Similarly, individuals who preferred online support over combined online and in-store support options were more likely to be living alone (large effect).

Conclusions: Financial toxicity appears to meaningfully shape hearing aid preferences, with cost-related concerns influencing willingness to adopt OTC devices and the type of support services desired. While affordability was an important factor, participants continued to value professional involvement and advanced features, suggesting a tension between financial constraints and preferred models of care. These findings underscore the need to consider financial burden when developing and promoting OTC hearing aid service delivery pathways to ensure equitable access without compromising patient preferences.

M41. Electrically Evoked Compound Action Potentials and Electrocochleography as Complementary Measures of Cochlear Health

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Background: Hearing performance of cochlear implant (CI) users relies on the condition of the auditory nerve. Animal studies have shown that electrically evoked compound action potentials (eCAPs) can be applied to assess this condition. Relative measures, obtained by comparing eCAPs to different stimuli, are strongly correlated with neural survival (Ramekers et al., J. Neurosci. 2015). Studies in humans demonstrated the value of such relative measures in their correlations with hearing performance (Zamaninezhad et al., Front Integr Neurosci, 2023). The presence of residual hair cells, which we assume is beneficial for neural survival, can be assessed with intracochlear electrocochleography (Giardina et al., Ear Hear, 2019). In an ongoing trial, we record eCAPs and ECoChG in CI recipients to examine the predictive value for hearing performance. Here we consider the relation between eCAPs and ECoChG.

Methods: Twenty subjects with severe sensorineural hearing loss, aged 34 to 82 years, received a CI (Flex28, MED-EL). Intraoperatively and four months postoperatively, eCAPs were recorded at each electrode to biphasic current pulses with varying interphase gaps (IPGs; 2.1 to 30 μ s) and current levels varying up to saturation (AutoART, MED-EL). Outcome measures include latency at maximum current levels, and several measures derived from sigmoid fitting of the amplitude growth function (AGF) such as maximum amplitude and the current level halfway the AGF, level50%. Relative measures were obtained by the difference between measures at IPG of 30 and 2.1 μ s. ECoChG was postoperatively performed to tones at 115 dB SPL at frequencies from 250 to 4000 Hz. Hearing performance was assessed by CVC in noise perception and the spectral-temporally modulated ripple test (Aronoff and Landsberger, JASA, 2013).

Results: Generally, maximum eCAP amplitudes varied between 200 and 1000 μV , typically with the higher values at apical electrodes. Cochlear microphonic (CM) could be recorded for most participants not only at low frequencies but occasionally also for 4000 Hz. Correlations between eCAP amplitude (averaged across electrodes) and CM amplitudes were not significant; neither did relative eCAP outcomes significantly vary with CM amplitudes. A within-subject analysis revealed strong correlations ($R > 0.5$) between eCAP and CM amplitude across the electrode array in five subjects, whereas weak or negative correlations were observed in nine subjects.

Conclusions: A lack of consistent correlations between eCAPs reflecting neural health and CM reflecting residual hair cells may be explained as follows. The CM mainly reflects surviving outer hair cells, while inner hair cells are more important for neurotrophic support of the SGCs. Alternatively, it is rather the supporting cells being relevant for neurotrophic support (Zilberstein et al., 2012) and hair cell survival does not mirror supporting cell survival that well. We conclude that ECochG and eCAP provide largely independent measures reflecting the health of different cochlear structures.

M42. The Acoustic Change Complex Compared to Onset Auditory Evoked Potentials in Cochlear Implant Users

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Background: The acoustic change complex (ACC) is a cortical potential evoked in response to a change within an ongoing sound stimulus. The ACC has been reported to correlate to speech perception in cochlear implant (CI) users and is therefore promising as an objective measure of auditory performance. Since processing of auditory changes is indispensable for speech perception, the ACC might be more predictive of speech perception than auditory potentials evoked in response to an onset stimulus. The aim of this study is to compare the ACC to onset potentials of the auditory cortex and brainstem, as objective measures of auditory performance in CI.

Methods: Thirteen bilaterally deaf and six single-sided deaf CI users aged between 18 and 63 years were included, all with unilateral CI (Cochlear Ltd) experience for at least one year (Van Heteren et al., 2022, Ear Hear 43:1783-1799). ACCs were evoked in response to frequency increases within pure tones. ACCs were compared to cortical auditory evoked potentials (CAEP) in response to onsets of pure tones, and to auditory brainstem responses (ABR) in response to biphasic pulses. ACC reference tones and CAEP tone stimuli had frequencies of about 500 and 2000 Hz corresponding to an apical and middle electrode respectively. The ABRs were evoked by biphasic pulses at the same apical and middle electrodes. Auditory performance tests included speech perception in quiet and in noise.

Results: Averaged across the two frequencies, both ACC amplitudes ($R = 0.51$ - 0.53 , $p = 0.019$ - 0.026) and latencies ($R = 0.51$ - 0.68 , $p = 0.003$ - 0.04) had strong correlations to speech perception in quiet and in noise. Onset CAEP amplitudes ($R = 0.51$ - 0.66 , $p = 0.002$ - 0.02) correlated to speech perception in quiet and in noise. ABR wave V amplitudes were only correlated to speech

perception in noise ($R = 0.55$, $p = 0.01$). Latencies of onset CAEP and ABR did not show such correlations.

Conclusions: The ACC has a stronger and more consistent relation to speech perception compared to onset CAEP and ABR. This can be contributed to the fact that the ACC reflects cortical processing of spectral modulations which are more important than sound onsets for perception of dynamic sounds as speech. The correlations found between speech perception tests and ACC amplitudes and latencies underlines the promising value of this test as an objective measurement of auditory performance in CI users.

M43. Auditory Processing of the Fast Noise Burst Sequences – An Electrophysiologic Perspective

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Background: The fast sequence of a short and a long noise burst (NB) separated by a short gap has been shown to evoke nonlinear transition of subcortical electrophysiological responses depending on their parameters (Burkhard et al. 2019). However, it was not clear whether, and how, this phenomenon is related to the perception of the discontinuity of such sound sequence. The present study focused on an analysis of electrophysiological responses and auditory perception of these NB sequences.

Methods: Electroencephalographic signals (EEG) were recorded from a selected skull positions using Ag-AgCl electrodes in a group of adult probands ($n=13$, age: range of 23-42 years) with normal hearing. Auditory responses were evoked by a sequence of two NBs separated by a short gap. Durations of the leading NB varies (5, 10, 30 ms), whereas durations of the trailing NB and gap are fixed (50 ms and 10 ms, respectively). Each NB has a 1 ms rising/falling phase. The stimuli were presented by Tucker-Davis Technology system RZ6 (TDT, Alachua, USA). Auditory perception of NB sequences with different parameters (durations of the first noise burst and following gap) were detected by answering a question, if probands hear one or two sounds after each presentation (min. 20 presentations). EEG signals were then recorded using TDT SI4 amplifier and RZ2 system (sampling rate of 24.414 kHz) to all three NB sequence conditions and separate NBs of corresponding durations (i.e. 5, 10, 30, and 50 ms) with repetition interval of 1.3 s with 10% jitter 300 trials for each stimulus condition were recorded.

Results: The combination of short leading NBs (5 and 10 ms) combined with the 10 ms long gap were usually perceived as one continuous sound, whereas leading NB of 30 ms duration were consistently perceived as two-sound sequence. The observed discrepancies in perception have been linked to differences in the evoked EEG responses within the early (0-100 ms) and late (150-350 ms) latency windows, corresponding to the auditory and perceptual processing, respectively.

Conclusions: Present results suggest electrophysiological biomarkers of diverse neuronal activations linked to specific auditory perception of fast sound sequences in EEG. Considering the importance of fast sound sequences in speech perception and their nonlinear processing nature, the results can provide insights into the temporal auditory processing underlying categorical speech perception.

M44. Differential Processing of Clear and Spectrally Degraded Sentences in the Human Cortex Revealed by Intracranial Electrophysiology

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Background: Cochlear implants (CIs) are the treatment of choice for severe to profound hearing loss. Despite progress in CI technology, there is considerable variability in outcomes. The function and plasticity of central auditory pathways contribute to this variability. While assessing cortical processing in CI users is methodologically difficult, spectrally degraded sounds presented to normal-hearing listeners can approximate the CI's input to the central auditory system. A previous intracranial electroencephalography (iEEG) study used spectrally degraded (noise-vocoded) speech tokens in a phonetic behavioral task (Nourski et al., 2024, *Front Hum Neurosci* 17:1334742). Differential responses to clear and vocoded stimuli could present as a “clear-preferred” or a less common “vocoded-preferred” pattern. This study sought to further characterize these differential responses using clear and vocoded sentences.

Methods: Participants were adult neurosurgical epilepsy patients undergoing chronic iEEG monitoring. Speech sentences (1.3-3.7 s duration) were degraded using a 3-band noise vocoder (Billig, Herrmann et al., 2019, *J Neurosci* 39:8679-89). Cortical activity was recorded using depth and subdural electrodes. Electrode coverage included superior temporal plane, superior temporal gyrus, dorsal and ventral auditory-related areas, prefrontal, and sensorimotor cortex. Analysis of iEEG data focused on event-related band power (ERBP) in canonical bands. Differences between responses to clear and vocoded sentences were established by cluster-based permutation tests.

Results: Differential responses (AEP, ERBP augmentation or suppression) to clear and vocoded sentences were broadly distributed in both hemispheres and could persist throughout the sentence duration. The vocoded-preferred pattern was less common than clear-preferred. Vocoded-preferred responses occurred bilaterally in the superior temporal plane, to a lesser extent in posterior regions of the superior and middle temporal gyri and inferior parietal cortex and were virtually absent in prefrontal cortex.

Conclusions: The results emphasize the role of auditory cortex and the dorsal stream in processing degraded speech. Clear-preferred responses are likely driven by spectral complexity and intelligibility. Vocoded-preferred responses may reflect processing demands associated with challenging listening conditions. These responses emerge at relatively early cortical processing stages and can engage the dorsal stream without extending into prefrontal cortex. Cortical regions that are differentially activated by clear and vocoded speech may have diagnostic and prognostic utility and present potential targets for neuromodulation-based CI rehabilitation strategies.

M45. Motor-Auditory Contributions to Speech: Trf Evidence Across Multiple Datasets and Implications for Imagined Speech

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Background: Producing speech engages interacting motor and auditory processes; During overt speaking, the cortex both issues articulatory/motor commands and receives auditory feedback from one's own voice. In mimed (silent) speech, the motor drive remains while external auditory feedback is absent. In listening, auditory input is present without articulation. Imagined speech engages internal motor and auditory simulations without overt movement or sound. We ask two questions: (i) Does neural activity during vocalized speech approximate an additive mixture of the mimed (motor-dominant) and listening (auditory-dominant) responses? (ii) Can activity across these sensory-motor modes help characterize, and even predict, the content of imagined speech? To test this, we analyze three modes of speech across complementary recordings: invasive sEEG (vocalized, mimed, imagined) and noninvasive EEG (speaking, miming, listening).

Methods: We analyzed two complementary datasets: (1) sEEG during vocalized, mimed, and imagined production (He et al., 2025); (2) dry-EEG during speaking, miming, and listening (dry sensors to reduce movement artifacts during speech/miming). Data were preprocessed and segmented by word and trial. Stimuli were represented with multi-band spectrograms (Chi et al., 2005). We fit forward TRFs (stimulus to neural) (Crosse et al. 2025) to reconstruct evoked responses and backward TRFs (neural to stimulus) to reconstruct the envelope/spectrogram.

Results: EEG (additivity with backward TRFs). Backward TRFs were fit separately for each sensory mode in the EEG data to reconstruct the audio spectrograms. We observed that the reconstruction for Speaking/Vocalized is well approximated by the sum of the reconstructions from Miming and Listening, supporting a superposition of motor and auditory components.

sEEG (transfer to imagination). We tested whether backward TRFs trained on vocalized speech could detect imagined words from sEEG. A backward TRF was fit to the vocalized responses to predict the audio spectrogram from sEEG, and the same model was applied to imagined responses for the same words. We observed a time-localized increase in the reconstructed spectrogram's energy consistent with word onset. Because imagery onset is uncertain, these increases were variably time-shifted across trials; ongoing analyses quantify and compensate for this latency variability.

Conclusions: TRF-based analyses on our EEG dataset provide converging evidence that overt speech responses can be approximated by additive contributions from motor and auditory components. Preliminary results from TRF analysis of the sEEG dataset show that models trained on vocalized speech transfer to imagined modes. This supports shared encoding across overt and covert speech and could offer a principled linear baseline for imagined-speech decoding, an essential step toward practical brain-computer interfaces for communication without articulation.

M46. Divergent Cortical Speech Tracking at the Cocktail Party: Preserved in Hearing Aid Users, Impaired in Cochlear Implant Users

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Background: Although modern hearing aids (HAs) and cochlear implants (CIs) effectively restore speech perception in quiet, understanding speech in complex acoustic environments remains a challenge. Competing talkers and noise demand high listening effort and can severely impede communication. Neuro-steered hearing prostheses, using EEG to decode attentional focus, offer a promising solution by enhancing the attended speech stream. However, developing such devices requires a deeper understanding of neural speech tracking in the hearing impaired population. While prior work investigated HA or CI users in isolation, a direct comparison is lacking. This study provides the first comprehensive, direct comparison of cortical speech tracking in HA and CI users, using a more ecologically valid paradigm with free-field stimulation and participants' clinical devices.

Methods: To this end, we collected EEG data from bilateral CI users (n=25), bilateral HA users (n=29), and age-matched typical hearing (TH) controls (n=29) during a selective attention paradigm. Participants listened to two-minute audiobook trials in both single-speaker and competing-speaker conditions. They were visually cued to attend to a target story. Post-trial comprehension questions and subjective listening effort ratings were used to quantify speech comprehension and perceived difficulty, across conditions and cohorts.

Neural tracking was assessed using a linear forward model to study the Temporal Response Function (TRF) to the speech envelopes. Moreover, a linear backward model served to examine the differences in reconstruction scores of the attended speech envelope and the decoding accuracy across cohorts. Statistical analysis comprised both parametric and non-parametric tests. If applicable p-values were corrected for multiple comparisons, using FDR-correction.

Results: Behaviorally, significant group differences emerged only in the competing-speaker condition. Self-reported listening effort showed a graded increase from controls to HA users to CI users (all $p < 0.01$). In contrast, objective comprehension was significantly impaired only for the CI group ($p < 0.001$), as both HA users and normal-hearing controls performed at ceiling.

The decoding accuracy of the attended speaker was significantly reduced for the CI user, compared to the HA and TH groups (all $p < 0.001$). This could be explained by higher reconstruction scores of the distractor envelope (all $p < 0.001$) that we observed for the CI group. Importantly, we found that the HA group exhibited cortical tracking and attentional modulation that were largely comparable to the TH group. This finding was supported by both forward and backward model analysis.

Conclusions: The first key take-away is that CI users exhibit significantly less neural speech tracking, and smaller attentional modulation of the speech tracking, in line with their behavioral difficulties in speech-in-noise comprehension. The second take-away is that modern HAs largely normalize cortical speech tracking. This suggests that their increased listening effort, as compared to typical hearing people, may have a different neural origin.

M47. Prevalence of Tinnitus According to Audiometric Profiles in a Population of Hearing-Impaired Subjects Who Are Candidates for Hearing Aids (n = 250,000)

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Background: We had access to a database collected by hearing aid suppliers from Amplifon retailers in France. We analysed more than 250,000 subjects with and without tinnitus. The main objective of this study was to assess whether the prevalence of tinnitus varied according to audiometric profiles.

Methods: For most subjects, we had audiograms, loudness discomfort levels, speech in silence and noise, and a visual analogue scale for tinnitus intensity and distress. All subjects were also asked whether they were or had been exposed to noise. The data were processed using principal component analysis and hierarchical clustering (PCA-HC) and logistic regression.

Results: Overall, the prevalence of tinnitus in this population is approximately 45%. This prevalence is very high, but it is probably due to the fact that the population is highly biased in that most subjects are hearing impaired. It is interesting to note that the prevalence of tinnitus follows a bell curve with age, peaking around 60 years of age. PCA-HC identified 14 subtypes of audiograms. Tinnitus increased with average hearing loss, but was highest for audiograms with steep slopes. Tinnitus prevalence is higher in the population of subjects who answered positively to the question relative to noise exposure. It is also enhanced in subjects with low loudness discomfort levels.

Conclusions: We discuss the results as a function of the tinnitus mechanisms.

M48. Responses of Inferior Colliculus Neurons to Notched Noises in Awake Mice: Putative Neural Correlates of Auditory Enhancement and Zwicker Tone

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Background: Sensory systems are well adapted to constantly changing statistics of the environment and to process specific spectral features of sounds, such as spectral notch (i.e. low energy frequency band) embedded in broadband stimuli. Spectral notches can be added to the stimulus spectrum due to filtering by the outer ear, and can be used as monaural cues related to head or pinna position for localizing sound sources. In addition, broadband sounds with spectral notch are known to produce auditory enhancement, a perceptual phenomenon in which a target within a spectrally notched masker can become salient if preceded by a copy of the masker. Notched noise can also produce an auditory illusion, called Zwicker Tone (ZT), which is perceived immediately after stimulation and whose pitch corresponds to the spectral notch.

Methods: The present study aimed to further investigate the mechanisms of auditory enhancement, including those of ZT, in the inferior colliculus of awake mice using glass electrode electrophysiology.

Results: We show that neural activity can be strongly suppressed during NN stimulation and enhanced immediately after NN stimulation. These effects depend on notch center frequency relative to the best frequency of neurons, stimulus level and notch width. Our results are consistent with the mechanisms described for post-inhibitory rebound in the central auditory system: NN could hyperpolarize the membrane potential, which can then activate several cationic conductances, leading to a rebound of neural activity.

Conclusions: We discuss auditory enhancement and ZT as collateral effects of an essential neural mechanism aimed at enhancing the central representation of acoustic spectral contrasts.

M49. Characterization of Masker Modulation Frequency Effects on Moc Activity in a Forward-Masking Paradigm

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Background: Although anatomical studies have established that the inferior colliculus (IC) provides a major projection to the medial olivocochlear (MOC) system, the physiological contribution of this pathway remains unclear. IC cells, sensitive to low-frequency modulation inherited from auditory-nerve (AN) responses, convey spectral information distinct from the level-driven input of the CN. We hypothesize that varying masker modulation frequency alters the IC input to MOC, independent of CN effects, with reduced MOC activity for frequencies outside the IC's modulation transfer function (MTF) range. This approach provides insight into IC contributions to MOC. In our previous work, we varied the modulation frequency of the probe while using the same broadband noise as the masker. Here, we instead vary the masker's modulation frequency while keeping the probe modulation fixed, ensuring the probe engages the same EFR-generating mechanisms across conditions so that differences can be attributed to the masker.

Methods: We recorded envelope following responses (EFRs) and transient evoked otoacoustic emissions (TEOAEs) simultaneously in awake chinchillas using a forward-masking paradigm. The goal was to test how masker modulation frequency influences the modulation enhancement effect, defined as an increase in EFR response in the masked compared to the unmasked condition. This effect has been reported in chinchillas (Farhadi et al., 2023) and humans (Bharadwaj et al., 2015), but here we specifically examine its dependence on masker modulation frequency. TEOAEs were measured before and after stimulation to assess cochlear gain changes (associated with MOC activation), and EFR responses were analyzed using phase-locking value (PLV) to quantify neural changes associated with MOC activation. We also tested whether effects differ between sedated and awake conditions to better link them to MOC mechanisms.

Results: We observed a broad reduction in TEOAE responses in the presence of a broadband noise masker, whereas responses in silence remained unchanged. For SAM tones, a larger decrease at the carrier frequency occurred for 103 Hz modulation (within the IC MTF range) compared to 303 Hz (outside the range). Similarly, EFR PLVs showed an increase in the first PLV peak under forward-masked conditions, with stronger effects at 103 than 303 Hz. Together, these results indicate a frequency-dependent enhancement of EFR responses, consistent with our hypothesis based on IC MTFs and supported by computational modeling.

Conclusions: This study establishes a forward-masking paradigm as a noninvasive approach to probe MOC activity as a function of masker modulation frequency. The design allows us to examine how IC input may shape MOC activity, complementing the better-studied cochlear nucleus pathway, and helps clarify the physiological contribution of IC projections to efferent control of the cochlea. Although developed in an animal model, the paradigm is noninvasive and therefore has potential for translation to humans, where within-subject normalization may be especially valuable.

M50. The Influence of Local Inhibition on Receptive Fields in the Inferior Colliculus

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Background: The inferior colliculus (IC) is the midbrain hub of auditory processing, receiving most of the ascending input from the lower auditory brainstem and providing the main ascending auditory input to the thalamocortical system. Many neurons in the IC exhibit complex receptive fields for sound features. While some of this complexity almost certainly arises from the combinations of ascending input that individual IC neurons integrate, a wealth of anatomical evidence indicates that the IC is rich in local circuits that may also play critical roles in shaping receptive fields. However, how these local circuits shape IC neuron responses to sounds remains unclear. Here, we are using in vivo electrophysiology in awake mice with optogenetic manipulations to examine how local inhibitory circuits shape IC neuron frequency tuning and selectivity for frequency modulated (FM) sweeps. We hypothesize that local inhibitory circuits sharpen IC neuron receptive fields such that disrupting local inhibition leads to broadened frequency tuning and reduced selectivity for FM sweeps.

Methods: To investigate how local inhibitory circuits in the IC impact sound responses, we are using inhibitory opsins to selectively inhibit IC inhibitory neurons while making in vivo recordings in the IC of awake, head-fixed mice. To selectively inhibit IC inhibitory neurons, we are injecting an adeno-associated virus containing a Cre-dependent construct encoding the inhibitory opsin eOPN3 into the IC of Vgat-Cre mice, in which Cre recombinase is expressed in GABAergic neurons. Two to six weeks later, we use 32-channel silicon probes with an integrated optical fiber to record from and deliver optical stimuli to the IC. During recordings, we assess frequency tuning using tone bursts ranging from 4 – 64 kHz at intensities from 0 – 70 dB SPL and assess FM sweep tuning using 70 dB SPL logarithmic FM sweep stimuli with speeds of 10 – 200 octaves/second and varying between the up and down direction. All sound stimuli are pseudorandomly presented under control conditions and in the presence of optical stimulation.

Results: Experiments for this study are ongoing. We expect to find that inhibiting local inhibition broadens frequency tuning and reduces FM sweep selectivity in many IC neurons.

Conclusions: Anatomical studies have repeatedly demonstrated that the IC is rich in local circuits, but due the intermixing of ascending and local inhibitory synapses in the IC, it has been difficult to directly test how local inhibitory circuits contribute to IC computations. We expect the results of this study will show that local inhibitory circuits critically shape the acoustic receptive fields of most IC neurons.

M51. Encoding of Amplitude-Modulated Stimuli in the Inferior Colliculus: Impact of Reduced Temporal Salience and Corticocollicular Projections

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Background: Subcortical auditory processing is shaped by ascending auditory projections but also descending, top-down influences. Top-down projections may compensate for degraded ascending acoustic information, seen with aging or in cluttered acoustic environments. One way to model temporal ambiguity is by reducing the modulation depth (m) of sinusoidally amplitude-modulated (SAM) stimuli. Prior work in the MGB revealed neurons that exhibited enhanced responses to repeated, temporally degraded signals in both young and aged rats. Building on these findings, we examined whether aging and reduced m alter single-unit responses to SAM stimuli in the rat inferior colliculus (IC), and how Corticocollicular (CC) projections contribute to shaping these responses.

Methods: We recorded IC single-unit activity in awake passively attending young (4–7 months) and aged (28–32 months) Fischer Brown Norway rats. SAM stimuli were delivered at 100% or at reduced m of 50% and 25%. An additional “Noisy SAM” condition was created by jittering the modulation envelope with low-pass (cutoff: 1 kHz) broadband noise (BBN). Modulation frequencies (Fmod) ranged from 2–1024 Hz, with the carrier set to BBN or to a unit’s CF. Stimuli were presented in blocked sequential trials or random across trials. To test whether reduced m increases reliance on CC inputs, we optogenetically suppressed CC terminals in young IC units by injecting an AAV-Arch-GFP construct into deep layers of primary auditory cortex. Following a 3-week period for the virus to express in the IC, simultaneous recording of IC units and light delivery was performed.

rMTF and tMTF were constructed based on discharge rate and synchrony plotted against Fmod for condition

Results: In vivo recordings revealed young IC units that often exhibited enhanced firing rates to sequentially present SAM stimuli at reduced m, while aged rat IC units could show enhanced response to sequentially presented stimuli both at 100% and at reduced m. Increased responses was observed for both age groups, though it was more prevalent in aged animals. In both groups, while some units showed decreased spiking when m was reduced, a substantial proportion displayed increased discharge rates at lower depths or Noisy SAM stimuli. Optogenetic blockade of CC inputs reduced the magnitude of these enhanced responses, often near the unit’s best modulation frequency (BMF). In some cases, CC blockade shifted the BMF. Temporal responses showed complex effects of CC blockade. Some IC units showed increased synchrony or expanded the range of Fmod showing synchrony, while other IC units showed reduced synchrony to CC blockade. This suggested complex modulatory effects of top-down input on temporal coding.

Conclusions: These findings support evidence that processing temporally degraded auditory signals, such as those with reduced m engages resources from Cortex to the IC, may be critical for perception during aging or in complex acoustic environments.

M52. Top-Down Signaling From the Medial Prefrontal Cortex to the Inferior Colliculus During an Auditory Oddball Paradigm

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Background: Detecting behaviorally relevant auditory information is essential for navigating in dynamic environments. Beyond processing of auditory information in the central auditory system, widespread projections from cognitive higher-order frontal regions support integrative, context-dependent control. The medial prefrontal cortex (mPFC) may be a key role, yet the extent of its influence on central auditory activity remains unclear. This study combined a three-class oddball, in which rats are required to differ between a behaviorally relevant target tone while ignoring distractor and standard tones, with simultaneous recordings from mPFC and the central nucleus of the inferior colliculus (ICc), to assess a potential interaction between these regions during auditory information processing in awake rats.

Methods: Following the training of rats (n = 8) and the stereotaxic implantation of chronic electrodes into the mPFC and ICc under anaesthesia, local field potentials (LFPs) were simultaneously recorded from both regions during an auditory three-class oddball paradigm (500 ms stimuli at 70 dB SPL), requiring behavioral response to a rare target tone (20%), while ignoring a frequent standard (60%) and a rare distractor tone (20%). The neural recordings were analyzed using event-related potentials (ERPs) and time-frequency analysis with a complex Morlet wavelet convolution (CMW), which was used to describe the temporal frequency patterns attributed to each tone. To compare the neural signal on a trial-by-trial basis, a Pearson cross-correlation was applied.

Results: ERPs showed an early ICc onset response for all tones (20 - 50ms post stimulus), characterized by enhanced beta (12-30 Hz) and gamma (> 30Hz) activity. In the mPFC, an early (100ms, N1) and late (300-450ms, P3) deflection was most pronounced for target. In the mPFC, for N1 low frequency activation (< 12Hz) appeared after all tones, whereas only targets evoked beta band responses. Interestingly, only after target, a second deviation in the ICc shortly following the mPFC N1 shared frequency patterns similar as the mPFC N1. Cross correlation indicated a switch in information flow from the mPFC towards the IC for target after mPFC N1, which was not observed for standard and distractor tones.

Conclusions: These findings suggest top-down processing from the mPFC to the IC for behaviorally relevant information. The similarity of spectral features of the mPFC N1 and subsequent ICc activation suggests a model in which prefrontal activity selectively amplifies auditory midbrain representations of behavioral relevant stimuli via top down pathways.

M53. Intracochlear Pressure Measurements During Bilateral and Bimodal Bone Conduction Stimulation

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Background: Clinical bone conduction hearing devices (BCHDs) have long been used for the diagnosis and treatment of hearing loss. Recently, BCHDs have also increased in popularity for personal audio applications, due to their ability to transmit sound to the inner ear without occluding the ear canal. BCHDs vibrate the head, thus both ears are stimulated when applying BC stimulation through even a single transducer. The relative timing and amplitude differences of sound arriving at each ear – known as transcranial delay (TD) and attenuation (TA) – are nonzero given a transducer placed on either side of the head, but are generally small and frequency-dependent. The introduction of a second BC stimulus (as in bilateral clinical BCHD fittings) and/or concurrent air conducted (AC) stimulation therefore leads to a complex superposition of signals, subject to a host of exogenous (e.g., stimulus, transducer, fitting) and endogenous (morphometric, physiologic) variables. Here, we report intracochlear pressure signals from a series of measurements in human cadaveric specimens designed to quantify effects of bimodal AC/BC and binaural BC interactions.

Methods: Whole cadaveric human heads were prepared bilaterally with canal wall up mastoidectomies and an extended facial recess approach, and suspended upside down via a loose fixation apparatus. Intracochlear pressure was measured in the scalae vestibuli and tympani with fiber optic pressure probes, concurrently with velocity of the cochlear promontory via laser Doppler vibrometry. Depending on the experiment, tone, click, or noise stimuli were presented through AC and/or BC modalities: AC stimuli were presented unilaterally via a loudspeaker (TDT FF1) adapted to the ear canal with PVC tubing. BC stimuli were applied to one or both mastoids with either pairs of RadioEar B81 transducers, or Brüel and Kjær mini-shakers (4810) coupled to bone anchored abutments at clinically typical bone-anchored hearing aid locations.

Results: Both bimodal (AC/BC) and bilateral (BC/BC) stimulation revealed complex frequency- and location-dependent interference that impacted the effective stimulation magnitude in each ear. Interference can result in unexpected and ostensibly undesirable consequences, including the genesis of nonmonotonic amplitude fluctuations with changing sound source location, and the introduction of spurious binaural cues, both predicted to disrupt sound localization. Further, the consequences of signal superposition differed between intracochlear (pressure) and extracochlear (promontory vibration) measures.

Conclusions: Results suggest that both bimodal AC/BC and bilateral BC stimulation produce complex, frequency- and location-dependent superposition of multipath signals. Modern signal processing approaches may provide avenues to mitigate the effective transmission of undesirable signals/components, provided that appropriate target signals (e.g., for cross-talk cancellation) can be identified and measured. Prospects for such interventions are considered.

M54. Signal to Noise: Sound Localization and Speech Perception During Bone Conduction Headset and Hearing Protector Use

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Background: Bone conduction (BC) hearing devices stimulate the cochlea via vibration of surrounding bone and through secondary and tertiary conduction pathways that are also engaged when the head vibrates – a mode of stimulation distinct from, but overlapping with, conventional air conduction (AC) stimulation. In both clinical (e.g., BC hearing aid) and non-clinical (e.g., BC

headset) contexts, intracranial BC signals may be superposed with AC signals at one or both cochleae, leading to complex interactions that may degrade transmitted acoustic information and constrain auditory performance. Here we provide an update on an ongoing series of psychophysical measurements of the impacts of BC headset use on two fundamental auditory tasks: sound source localization and speech perception. In a subset of conditions, subjects also wore earplugs to capture performance in contexts (e.g., military operational settings) in which hearing protection and access to communications signals are both required.

Methods: In a darkened anechoic chamber, human subjects performed two different tasks: In a localization task, subjects were instructed to localize (via head turn) brief noise bursts presented through loudspeakers while listening to a concurrent speech signal presented through a BC headset. The target AC signal was varied in azimuth, elevation, amplitude, and timing relative to the BC stimulus. Subjects completed the task with open ears and with bilateral earplugs. AC-alone (target-only) performance was also assessed. In a speech perception task, subjects completed a BC-adapted version of the Modified Rhyme Test. Brief sentences with embedded keywords were presented through the BC headset while spatially diffuse pink noise of varied amplitude was presented from eight loudspeakers surrounding the subject (i.e., via AC). Subjects again completed the task with and without bilateral earplugs.

Results: Interactions of AC and BC signals at the cochleae are predicted to result in an AC-BC sum with amplitude and phase characteristics that differ from those of either component signal. However, the extent of interaction – and associated impact on performance – depends directly on the effective spectrotemporal overlap of the component signals, which can be limited by attributes of the sound sources themselves (including relative amplitudes) and especially by filtering characteristics of the BC transducers in use. The concurrent use of earplugs degrades the localization of externally presented sounds, but significantly improves the perception of BC-transmitted speech, ostensibly due to both passive attenuation of external noise and occlusion effect-based boosting of the speech signal.

Conclusions: BC headsets are often used to transmit personal audio or communications signals in settings that also require situational awareness for safe navigation and/or tactical decision-making. In such contexts, the definitions of “signals” and “noise” can change unpredictably. Future work will consider strategies to mitigate masking of critical AC spatial cues by BC headset audio.

M55. Bimodal Localization and Lateralization Response Distributions in Some Normal-Hearing Listeners

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Background: It is assumed that binaural processing is exquisitely sensitive and accurate in normal-hearing individuals, which supports superb horizontal-plane sound-localization abilities and a remarkable ability to perceive speech in noisy cocktail-party-like environments. This includes the ability to perceive space continuously. When a listener has hearing impairment or uses a hearing-assistive device, there can be a breakdown of these abilities. For example, the listener might respond that the sound is in the left or right hemifield, without any fine ability to determine sound location. In other words, the listener produces a bimodal response distribution

where responses are primarily to the left or right, with few responses directly in front of the listener or in the center of the head. Such response patterns appear to typically have subtle effects in typical reporting of results, meaning they are arguably hidden in most reports. The purpose of this study was to make a rough estimate of often adults with assumed normal hearing respond with a bimodal distribution rather than the expected uniform distribution in laboratory psychoacoustic experiments.

Methods: We did a retrospective analysis of data from five localization and intracranial lateralization experiments. Critically, individual responses were visualized. Several summary metrics were compared to determine if they could be used to identify a bimodal response distribution. This included the root-mean-squared error and slope of a functional fit to the data.

Results: In our retrospective analysis, we found multiple cases of normal-hearing listeners with bimodal response distributions that were previously not noted, particularly in intracranial lateralization studies. In some cases, group mean data hid the presence of bimodal localization and lateralization response distributions. Plotting individual responses as well as response distribution histograms best revealed bimodal response patterns in individuals. Individual mean data for each stimulus location (source angle for localization and interaural difference applied for lateralization) obscured those with bimodal response distributions; these responses had larger error bars and steeper slopes as a function of azimuth for conditions that should be perceived from straight ahead or centrally. Root-mean-squared errors were larger for bimodal response distributions, but the values could be similar to other types of error patterns (e.g., a systematic bias to one side).

Conclusions: Bimodal response distributions were surprisingly common. They occurred more often in lateralization experiments, but also happen in localization experiments and were sometimes stimulus specific. It is unclear if these normal-hearing listeners had some unidentified pathology or if bimodal response distributions are typical. Moreover, the prevalence may differ across studies for an unknown reason. These results should be carefully considered when researchers attempt to identify abnormal binaural processing in clinical populations with hearing impairment or other medical conditions.

M56. ITD Sensitivity in Normal Hearing: The Role of Age and Temporal Stimulus Structure

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Background: Sound localization is based on two binaural cues: interaural time difference (ITD) and interaural level difference (ILD). In normally hearing (NH) adults, the ITD sensitivity threshold for 250 Hz is approximately 25-80 μ s. In comparison, little research has been conducted on ITD sensitivity in children. Sensitivity to binaural stimuli can be influenced by

various factors such as listening experience, general and central developmental status, or the type of sound stimulus. This study investigated the development of ITD sensitivity with increasing age and its dependence on stimulus parameters.

Methods: Eleven NH children ($\bar{X}$ 9.05 years old) and 10 NH adults ($\bar{X}$ 34.03 years old) were tested on sound lateralization using a 2-alternative forced choice gamified setup. Acoustic stimuli were 0.5 second long tone bursts at 250 Hz with a rising and falling slope of 4 ms (onset and ongoing ITD information) or 100 ms (ongoing ITD information only) and were presented via headphones. All subjects were tested for different ITDs of $\pm\{25, 50, 100, 200, 400, 800\}$ μ s. The ITD sensitivity thresholds (just-noticeable differences, JNDs) were calculated for the two ramp durations using psychometric functions.

Results: Compared to NH children ($\bar{X}$ 93.61 μ s), NH adults showed significantly better ITD JNDs ($\bar{X}$ 64.79 μ s) for both stimulation conditions. In NH children, ITD sensitivity showed a positive correlation with age under both conditions, indicating an improvement with increasing development of the auditory pathway. Notably, both cohorts showed significantly better ITD JNDs when the acoustic stimuli were presented with a ramp duration of 4 ms (children: $\bar{X}$ 92.07 μ s, adults: $\bar{X}$ 55.78 μ s) rather than 100 ms (children: $\bar{X}$ 113.89 μ s, adults: $\bar{X}$ 73.28 μ s).

Conclusions: In conclusion, auditory networks for ITD-based spatial hearing are still maturing in early childhood, resulting in poorer ITD sensitivity < 9 years compared to mature adult networks. Regardless of developmental stage, better ITD sensitivity was achieved with acoustic stimuli containing onset ITD information (4 ms ramp duration), confirming the importance of onset ITD sensitivity for optimal spatial hearing.

M57. Developmental Plasticity In Barn Owl ITD Circuits

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Background: Barn owls experience increasing interaural time differences (ITDs) during development, because their head width more than doubles in the month after hatching. We hypothesized that their ITD detection circuit might be modified by experience. To test this, we raised owls with unilateral ear plugs then measured the ITD representation in the brainstem nucleus laminaris (NL) when the birds were adult. The ITD circuit is composed of delay line inputs from the nucleus magnocellularis (NM) to the coincidence detectors in NL, and we predicted that compensatory plastic changes would lead to shorter delays in the axons from the manipulated ear. In owls that received ear plugs starting around P14, the maps of ITD shifted in the predicted direction, but only on the ipsilateral side, and only in those tonotopic regions that had not experienced auditory stimulation prior to insertion. The contralateral maps did not change. We recorded a frequency imbalance of the inputs from NM to NL which could be large enough to affect ITD tuning but was insufficient to account for all measured ITD shifts (Carr et al, 2024). We therefore examined the anatomical consequences on this circuit of reducing afferent auditory activity.

Methods: Experiments were conducted at the University Maryland, where 4 owls were raised with a unilateral earmold plug from P14 to 3 months. For immunohistochemical studies of GABAergic terminals, owls were perfused and sectioned at 30 μ m. Antisera against vesicular

glutamate Transporter (VGAT) were used at 1:1000, and terminals quantified using Neurolucida. Axon diameters were measured in EM perfused material.

Results: We examined the effects of unilateral earplug raising by measuring cell sizes, NM axon diameters and GABAergic innervation in adult birds after plug rearing. GABAergic inputs originate from the superior olive and mature in a low to high best frequency gradient in both NM and NL in the first month post-hatch. In ear plug raised birds, GABAergic terminal density was unchanged in NL both ipsi- and contralateral to the ear insert. In NM, VGAT immunohistochemical label on the earplug side revealed higher densities of GABAergic terminals in the high best frequency tonotopic regions that had not experienced auditory stimulation prior to plug insertion. VGAT label in the contralateral NM was indistinguishable from controls.

Conclusions: Thus, altered auditory input during development is associated with long-lasting changes in the inhibitory elements of the circuit for computation of ITD.

M58. Spatial Release From Speech-On-Speech Masking in Reverberation Evaluated Using the Portable Automated Rapid Test

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Background: This study used the Portable Automated Rapid Testing (PART) system to examine the effect of reverberation on spatial release from masking (SRM) for speech stimuli in a multi-talker environment. To examine the informational masking aspects of SRM, the experiment used a closed-set corpus (Coordinate Response Measure, CRM) and symmetrically placed maskers, reducing the energetic benefits of target-masker separation

Methods: Ten young adults with normal hearing (ages 20-32) completed a speech-on-speech recognition task in a sound-attenuating booth, using calibrated headphones and a tablet running the PART system. On each trial three different sentences with the structure “Ready [callsign] go to [color] [number] now” were presented simultaneously and the listener’s task was to identify the color and number in the sentence containing the callsign “Charlie”. The three sentences were spoken by three different male voices and differed in the callsigns, colors, and numbers. The target’s position was always simulated directly ahead of the listener, and the maskers could be either co-located with or spatially separated from the target (simulated at +/- 45° azimuth). The simulated environment was either anechoic or reverberant, generated using a modified image source model simulating a 10 × 10 m room with a reverberation time of 0.4 s, with the subject placed slightly off center to enhance realism of the simulation. Testing employed an adaptive scan procedure consisting of five scans (two non-adaptive, three adaptive), each with nine steps and a three-error termination rule. Trial-level data were analyzed with a general linear model to estimate the slope and 50% threshold of the psychometric function in each condition. Errors were categorized as intrusions if the response matched the color and/or numbers from the masker(s).

Results: SRM was 8.5 dB in the anechoic environment, reduced to only 1.1 dB in reverberation. This was driven mainly by an increase in separated reverberant thresholds (8 dB higher on average than separated anechoic thresholds), whereas co-located thresholds only increased by 0.5 dB in reverberation. The slopes of the psychometric curves were comparable in the co-located conditions (anechoic: 13.4 %/dB, reverberant: 13.3 %/dB). In the separated conditions, slope was shallower overall, particularly in the anechoic condition (anechoic: 8 %/dB, reverberant: 12 %/dB). Most errors were categorized as intrusions, accounting for approximately 85-90% of errors in most conditions, while only accounting for approximately 75% of errors in the separated anechoic condition

Conclusions: The study showed that PART can be used to obtain measures of SRM consistent with published approaches. Results suggest, in scenarios with high informational masking, the benefit of spatial separation can be dramatically reduced in reverberant vs anechoic environments, possibly due to increased difficulty segregating the target from maskers in reverberation.

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M59. FMRP Regulates Central Projection Assembly and Synaptic Maturation of Auditory Ganglion Neurons

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Background: The molecular mechanisms guiding the maturation and central projection of auditory ganglion (AG) neurons remain poorly defined. Fragile X messenger ribonucleoprotein (FMRP), a conserved RNA-binding protein essential for neural circuit development, is highly expressed in AG neurons across vertebrate species. However, its role in AG neuron maturation and connectivity remains unknown.

Methods: FMRP expression in chicken AG neurons was selectively reduced using drug-inducible, cell group-specific knockdown in live embryos. Effects on AG axon targeting and the structural and functional formation of presynaptic endbulb terminals within the primary cochlear nucleus—nucleus magnocellularis (NM) in birds—were assessed via axon tract tracing, 3D reconstruction, and FM dye imaging. Molecular players and mechanisms were identified using immunocytochemistry and RNAscope. To evaluate evolutionary conservation, wild-type and *Fmr1* knockout mice were analyzed for endbulb terminal phenotypes.

Results: FMRP knockdown in developing AG neurons led to frequent axon targeting failures onto NM neurons, most pronounced during the axon guidance window. During this period, NM-derived *Sema3A* normally induces AG growth cone collapse; this response was blunted when FMRP was deficient. Mechanistically, FMRP loss caused nuclear accumulation of *plexinA1* mRNA, reducing its cytoplasmic and axonal levels and impairing *Sema3A* responsiveness. In axons that successfully targeted NM neurons, structurally intact terminals were formed, but their function was compromised. This deficit was linked to delayed upregulation of synaptotagmin 2 (*Syt2*), a primary calcium sensor on synaptic vesicles. FMRP stabilized *Syt2* mRNA without

affecting its nuclear export, and knockdown reduced Syt2 transcript abundance. Fmr1 knockout mice exhibited similar deficits in Syt2 expression during the circuit assembly window, confirming conservation of FMRP function.

Conclusions: This study identifies two distinct FMRP-mediated mechanisms critical for AG neuron central projection and synaptic maturation. During axon targeting, FMRP facilitates nuclear export of plexinA1 mRNA, enabling proper Sema3A signaling and target recognition. During presynaptic development, FMRP stabilizes Syt2 mRNA, ensuring timely synaptic functionalization. These findings establish FMRP as a cell-autonomous regulator that orchestrates discrete stages of synaptogenesis via differential regulatory mechanisms.

M60. In Vivo Imaging of the Auditory Pathway in Mice Using Bundle Fibers

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Background: Physical or chemical stimuli from the environment are converted into electrical signals by peripheral sensory receptors. These signals then ascend through successive synapses in the nuclei of sensory pathways, ultimately reaching the sensory areas of the cerebral cortex. Once in the cerebral cortex, it is known that sensory information is integrated in distinct sensory areas corresponding to different modalities, or is modified by memory and emotion in structures like the hippocampus and amygdala before being recognized. In auditory processing, evoked responses in relay nuclei to sound stimuli have been studied in various vertebrates using electrophysiological technique. Recently, methods using optical imaging techniques—such as flavin fluorescent proteins, endogenous signals, or calcium-sensitive fluorescent proteins—to observe activity in areas like the auditory cortex have become widely employed. However, optical imaging can only observe the brain surface and to a certain depth. Furthermore, electrophysiological methods make it difficult to observe function and behavior in the awake state. Therefore, we attempted to observe deep brain neural activity or morphology in the awake state by implanting extremely fine optical fibers into the central nervous system.

Methods: Thy1-EGFP mice (genetically modified mice expressing GFP under the control of the Thy1 gene promoter in neurons of the hippocampus, amygdala, and cerebral cortex layer 5, used to observe neuronal morphology) and wild-type mice were used as experimental animals. In wild-type mice, to observe neuronal activity, the green calcium-sensitive fluorescent protein GFP-based Calcium Calmodulin probe (hereafter GCaMP: a green fluorescent calcium sensor genetically engineered from GFP, a green fluorescent protein used for fluorescent labeling of cells and biomolecules) was expressed in deep brain nuclei using an adeno-associated virus. A bundle fiber (diameter 0.6 mm) consisting of a bundle of microfibers was used as the optical fiber. Using these mice, the optical fiber was carefully implanted through the dorsal scalp under anesthesia. The objective lens and optical fiber were connected via a rotary joint.

Results: Observation using a high-brightness LED as the excitation light source allowed visualization of fluorescence from deep brain nuclei through the optical fiber. Changing the depth resulted in spatially distinct fluorescence patterns within the nuclei. Furthermore, time-lapse imaging of the deep brain regions of wild-type mice expressing GCaMP in excitatory neurons revealed temporal changes in GCaMP fluorescence intensity.

Conclusions: These results suggest that using bundle fibers may enable in vivo observation of the morphology or activity of deep brain neurons. Furthermore, since the same bundle fiber can transmit light bidirectionally, it is considered applicable to experimental systems using optogenetic techniques such as channelrhodopsin to evoke or suppress neuronal activity.

M61. Noise-Induced Reduction and Early Recovery of SPN OFF Responses

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Background: Neuronal circuits exhibit a remarkable capacity for plasticity in response to altered sensory input. The temporal dynamics and cellular mechanisms underlying this plasticity are highly heterogeneous and seemingly specific to individual circuits. Excessive noise exposure leads to damage of peripheral auditory structures, such as cochlear hair cells and auditory nerve fibers, resulting in reduced afferent input to downstream structures and initiating a cascade of long-lasting compensatory changes in central auditory circuits. Among these changes, increased neuronal excitability, elevated spontaneous firing rates, and generalized enhancements in neural gain were reported in multiple structures from the cochlear nucleus to the auditory cortex. However, these findings have primarily involved neurons that are responsive to sound onset. In contrast, little is known about the impact of noise-induced hearing loss on neurons that are selectively activated by sound offset and predominantly driven by inhibitory synaptic input.

Methods: To address this gap, we investigated the effects of noise exposure on the intrinsic membrane properties, synaptic input patterns, and sound-evoked activity of neurons in the superior paraolivary nucleus (SPN)—a population of neurons specialized for encoding sound offset. We employed a combination of immunohistochemistry, in vitro whole-cell patch-clamp recordings from acute brain slices, and in vivo single-unit recordings to assess the mechanisms underlying offset response generation in SPN neurons following moderate noise exposure. The noise paradigm was designed to induce threshold shifts of up to 40dB, and neural responses were assessed at two time points: immediately after exposure (0 hours) and 24 hours post-exposure.

Results: Consistent with previous reports, we observed increased intrinsic excitability in SPN neurons at 24hrs following noise exposure. However, contrary to the prevailing hypothesis of reduced inhibition as a driver of central gain, we found that inhibitory input to SPN neurons was not diminished but rather enhanced. This observation was confirmed across multiple methodological approaches and was associated with preserved suprathreshold offset responses exhibiting spectral and temporal tuning properties comparable to those observed in control animals.

Conclusions: Together, these changes contribute to the recovery of suprathreshold sound-offset responses 24 hours after noise exposure. Notably, neurons in the lateral superior olive—which are anatomically close to the SPN and also receive glycinergic input from the medial nucleus of the trapezoid body—exhibited reduced mIPSC frequencies following noise exposure. This finding supports the idea of a circuit-specific compensatory mechanism that helps sound-offset (OFF) encoding in the face of peripheral auditory damage.

M62. Auditory Deficits Following Continuous Noise or Single Blast Exposure

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Background: Service members are often exposed to harmful acoustic environments—from prolonged moderate-level noise (e.g., living and working on aircraft carriers) to brief high-intensity blasts (e.g., improvised explosive devices). Understanding how these different noise exposures affect auditory processing is critical. In this study, we investigated peripheral and central auditory functional deficits following continuous noise exposure simulating aircraft-carrier conditions or a single blast exposure simulating mild-to-moderate traumatic brain injury (TBI) using a chinchilla model.

Methods: For continuous noise, awake chinchillas were exposed for 1, 2, or 4 consecutive weeks (40 hours/week) at 87.5 dBA adjusting for cross-species middle-ear differences. For blast, anesthetized chinchillas were exposed to a single (1) blast at 150 kPa reflected peak overpressure with bilateral hearing protection (i.e., ear plugs). We employed a multi-metric auditory framework including tympanometry, wide-band middle-ear muscle reflex (WB-MEMR), otoacoustic emissions (OAEs; swept distortion product, DP; swept stimulus frequency, SF; transient evoked, TE), auditory brainstem response (ABR), and envelope frequency response (EFR) to amplitude-modulated stimuli. Electrophysiology (ABR and EFR) and blast exposures were performed under anesthesia using xylazine (2 mg/kg) and ketamine (20 mg/kg). Peripheral measures (tympanometry, WB-MEMR, and OAEs) were collected with fully awake but restrained animals.

Results: Continuous Noise Exposure (1, 2, or 4 weeks post-exposure): Reduced DPOAEs by 5–20 dB across all frequencies, SFOAEs decreased by 5-10 dB at mid-to-high frequencies, and diminished WB-MEMR strength as early as one week and remained up to four weeks. ABRs revealed mild-to-moderate (5–30 dB) threshold shifts across all frequencies while EFRs showed degraded temporal encoding occurs at high modulation frequencies as early as one week. However, ABR thresholds (1 and 2 kHz + click) and temporal encoding showed signs of recovery by week four. Single Blast Exposure (0.5, 2, 4, and 8 weeks post-exposure): No effects were observed for DPOAEs, SFOAEs, and WB-MEMR—as expected given bilateral protection with earplugs. Hearing thresholds were mostly unaffected except at 8 kHz with 5-10 dB shifts appearing until week four. Temporal encoding was degraded as early as three days post-exposure followed by hypersensitivity on week four that remained until week eight. Both continuous-noise and single-blast exposure showed mixed TEOAE effects across frequencies.

Conclusions: Together, these findings demonstrate continuous noise and single blast exposures have unique auditory deficits. Continuous noise at moderate levels caused persistent hearing loss and cochlear damage accompanied by transient temporal processing deficits. On the other hand, single blast exposure at intense levels led to minimal peripheral deficits but persistent temporal processing deficits. Our study shows the importance of a multi-metric auditory framework to highlight potential differences in auditory deficits that can be used to differentiate noise- and blast-induced hearing loss while helping to develop effective individualized treatment plans.

M63. Presynaptic Sodium Leak Channel NALCN at the Calyx of Held Synapse

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Background: At chemical synapses, the invasion of an action potential activates voltage-gated Ca²⁺ channels, leading to highly localized, transient Ca²⁺ influx that triggers vesicle fusion and neurotransmitter release. Although the large Ca²⁺ transients are required for rapid phasic exocytosis, low concentrations of basal Ca²⁺ can modulate release via vesicle recruitment, priming and sensitization, and subtle changes in basal Ca²⁺ levels alone can profoundly alter neurotransmitter release and synaptic strength. The Ca²⁺ channels mediating action-potential triggered release have been extensively studied. However, the ion channels that set the resting Ca²⁺ levels in nerve terminals remain enigmatic.

Methods: Brain slices from wildtype (WT) and NALCN conditional knockout (cKO) mice were harvested for electrophysiological recordings, imaging, and immunohistochemistry.

Results: We found that NALCN is expressed in calyx of Held terminals, where it not only controls the resting membrane properties but also regulates cytosolic Na⁺ level to drive glutamate uploading into synaptic vesicles. Surprisingly, presynaptic NALCN, unlike that in its recombinant counterpart, exhibits previously unappreciated Ca²⁺ permeability. NALCN is tightly coupled with active zone proteins and occupies transsynaptic nanocolumns between postsynaptic glutamate receptors and presynaptic release sites, directly mediating Ca²⁺-dependent synaptic transmission. Pharmacological blockade of NALCN or selective genetic deletion of NALCN from nerve terminals ablates these effects, weakening synaptic strength and neurotransmission fidelity.

Conclusions: NALCN is a key contributor to the resting membrane properties of central mammalian nerve terminals. It regulates neurotransmitter release via two distinct pathways: by modulating presynaptic resting Na⁺ and Ca²⁺ concentrations, NALCN regulates vesicular glutamate transport and vesicle release probability, ultimately maintaining the fidelity of neurotransmission across the central synapses.

M64. From Compression to Periodicity: Cochlear Influences on Suprathreshold Neural Encoding

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Background: The nonlinear mechanical properties of the cochlea, mediated by the outer hair cells, offer low-level amplification and mid-level compression of basilar membrane vibrations, which contribute to the broad dynamic range of human hearing. While these nonlinearities are often assumed to be uniform across normal-hearing listeners, there is increasing evidence for individual differences in both low-level gain and compressive growth. However, the extent to which these differences impact the neural encoding of sustained envelope periodicity, important for speech understanding, remains poorly understood. The envelope following responses (EFRs) serve as a reliable indicator of subcortical synchronization to amplitude modulation (AM). We hypothesize that cochlear compression will modulate EFRs measured at shallow modulation depths (e.g., 40% AM), making them more sensitive to individual differences than EFRs at deep

depths (100% AM). Specifically, individuals with weaker cochlear compression—indicated by steeper DPOAE input-output slopes—will exhibit stronger EFRs at shallow AM, while EFRs at deep AM remain robust and insensitive to compression. Studying the relationship between cochlear compression and EFRs could improve synaptopathy diagnosis by revealing how peripheral mechanical deficits influence neural encoding.

Methods: Envelope following responses and DPOAE were obtained from thirty normal-hearing adults (N=30; mean age=21.7 years, SD=3.6; 29 females). EFRs were elicited with a sinusoidally AM 4 kHz carrier at 101.56 Hz, with modulation depths of 40% and 100%. Wavelet analysis was used to derive EFR metrics, including signal-to-noise ratio (SNR) and phase-locking value (PLV). DPOAEs were obtained by varying the f2 level (L2) from 20 to 70 dB SPL and calculating the f1 level (L1) using the “scissor paradigm” ($L1=0.4 \times L2+39$). DPOAE I/O functions were fit with a two-segment piecewise linear regression model, obtaining slopes for the linear (S1) and compressive (S2) regions, as well as compression thresholds. Pearson's correlations were calculated between DPOAE slopes (S1, S2) and EFR metrics (SNR, PLV) across modulation depths.

Results: At shallow modulation depths (40%), the linear slope (S1) showed a significant positive correlation with PLV ($r=0.456$, $p=0.002$), but no correlation was observed at 100% modulation depth. For compressive slope (S2), a significant positive correlation was found with both SNR ($r = 0.326$, $p = 0.022$) and PLV ($r = 0.527$, $p < 0.001$) at 40%. However, no significant correlations were observed between S2 and PLV at 100% modulation depth. These findings revealed that cochlear compression contributes to subcortical envelope encoding at shallow modulation depths, while its influence is minimal at deep modulation depths.

Conclusions: Our findings demonstrate that individual differences in cochlear nonlinearities, particularly compressive gain, influence subcortical encoding of temporal envelopes at shallow modulation depths. These results indicate that EFRs reflect not only neural integrity but also peripheral cochlear mechanics, providing insight into the variability of envelope encoding among normal-hearing listeners.

M65. In Vivo Single-Cell Electroporation of Physiologically Identified Neurons in the Mouse Cochlear Nucleus

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Background: The cochlear nucleus (CN) serves as the initial station in the central ascending auditory pathway, processing acoustic information through its diverse cell types. Although single cell physiological and labeling studies provide extensive insights into structure-function relations of CN neurons, understanding the input-output transformation of individual neurons requires a comprehensive knowledge of connectivity map between single CN neurons and its presynaptic inputs. Single-cell initiated retrograde viral tracing from cells recorded in vivo offers a promising approach to address this question. The first step for this approach requires transfecting plasmids into a recorded neuron, achieved through in vivo single-cell electroporation or whole-cell recording. While this technique has been applied in several brain regions, its use in the auditory system remains unexplored. In this study, we investigate the feasibility of single-cell electroporation with simultaneous in vivo recordings in the mouse CN.

Methods: Anesthetized C57BL/6 mice (P35–P82), induced with zoletil-xylazine mixture (40/12 mg/kg), was used in this study. Lateral cerebellum was aspirated to expose the CN. A glass micropipette (tip resistance 5–8 MΩ) filled with an internal solution containing fluorescent protein plasmids (200ng/μL pAAV-CAG-GFP) was used for blind in vivo cell-attached recording. Sound stimuli consisted of pure tones, click trains, noises, AM tones, and harmonic complex tones to characterize single-neuron response to sounds. After recording, high-voltage pulses (–6 V, 0.5 ms, 50 Hz for 1 s) were applied through the same electrode, and the electrode was then carefully withdrawn. The mouse was allowed to recover for seven days before examination of transfection results.

Results: We found that single-cell electroporation provided more specific neuronal labeling than conventional biocytin labeling. GFP expression was observed in single CN neurons without apparent contamination due to pipette leakage. While consistent labeling remains a challenge, in cases where successful transfection is achieved, the soma and dendrites could be clearly labeled, and the axon could be traced for up to a several millimeters. In a few cases, the soma was absent despite successful labeling of dendrites and axon. Electrophysiological and morphological data could be integrated: for instance, a dorsal CN neuron responding with long first spike latency to its best frequency projects its axon through the dorsal acoustic stria, consistent with features of a fusiform cell. Both principal cells and interneurons could be labeled, including principal neurons such as octopus cells, fusiform cells, and interneurons such as vertical cells and cartwheel cells.

Conclusions: We demonstrate the feasibility of in vivo single-cell transfection with electrophysiological recordings in mouse CN neurons. Further refinement of the protocol, aimed at a more consistent transfection rate, will establish a foundation for single-cell initiated retrograde viral tracing to investigate the functional organization of the auditory brainstem in finer detail.

M66. A Comparison of Auditory Brainstem Responses to Diverse Stimulus Conditions in Rhesus Macaques

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Background: Nonhuman primates (NHPs) are a critical translational model for human hearing, with cochlear mechanics, brainstem and cortical organization similar to those of humans. Crucially, macaques have similar noise exposure susceptibility to humans, and support human-like perceptual and behavioral paradigms, enhancing the translation of both physiological and functional outcomes. In addition, NHPs permit controlled acoustic exposures and post-exposure intervals prior to histology. Together, these features make NHPs uniquely suited for auditory-system studies, particularly of noise injury and its brainstem correlates. However, normative non-invasive physiological measures spanning multiple stimulus classes are scarce in this species. To remedy this, we measured physiological responses to a wide range of stimuli.

Methods: Anesthetized young adult rhesus macaques (*Macaca mulatta*, age 4-7 years, n=16, 8 female, 8 males), were tested using non-invasive physiological assays, including auditory brainstem responses (ABRs) and Distortion Product Otoacoustic Emissions (DPOAEs) in a sound-treated

RF-shielded booth. Anesthesia was induced with ketamine and midazolam and maintained with isoflurane. Eight physiological tests are reported: DPOAEs (1-32 kHz), tones (0.5–32 kHz), chirps and clicks presented at various rates (27.7-200 /s), click and chirp pairs with varying inter-stimulus intervals (1-10 ms). For each, we extracted standard wave metrics (Waves I/II/IV amplitudes and latencies) and quantified ear-wise asymmetries.

Results: Thresholds to tones showed the expected U-shaped frequency dependence, with no sex-related differences. Click and chirp thresholds also showed no sex related differences. Inter-ear threshold differences were ≤ 10 dB SPL for most subjects, with no significant sex- or frequency-related bias. Paired-stimuli conditions exhibited canonical temporal-recovery signatures: reduced response amplitudes and prolonged latencies compared to the first stimulus response at short inter-stimulus intervals, with responses more similar to the first stimulus at longer intervals. Rate-manipulation experiments produced rate-dependent reductions in amplitude and latency increases at higher stimulus rates. Across ears, per-wave response amplitude correlations were stronger for closer tone frequencies and for chirp–click comparisons. Latency correlations were weaker and sparser than amplitude correlations.

Conclusions: Together, these results provide a comparative reference for macaque ABRs across complementary stimulus classes—covering thresholds, ear asymmetries, and temporal-recovery functions—and furnish benchmarks to support longitudinal studies of auditory brainstem physiology in a translational primate model. These data form pre-exposure baselines for chronic noise exposure in the same cohort. By spanning complementary stimulus classes and including DPOAE, this baseline enables sensitive detection of exposure-related changes, including subtle synaptic / neural deficits that may precede threshold shifts, through shifts in wave-I/II/IV amplitudes and latencies, altered temporal recovery functions, and rate-dependent modulation.

M67. Neurons of the Small Cell Cap

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Background: Auditory processing requires the brain to extract a wide breadth of information from acoustic signals. This is accomplished in part by heterogeneity of neurons in the cochlear nucleus (CN), an obligatory relay point of auditory nerve activity. Heterogeneity of auditory neurons is necessary to extract specific features, such as sound intensity, from cacophonous auditory nerve activity. Therefore, it is critical to understand the role of each type of neuron in CN processing to understand how sound features are initially represented in neuronal activity. In vivo recordings from the small cell cap (SCC), a region just ventral to the granule cell domain, showed that these neurons encode amplitude modulations and sound intensity with higher fidelity than other CN neurons. However, little is known about the intrinsic properties of these neurons, nor what function they provide within the circuit. SCC receives exclusively high threshold/low spontaneous rate inputs thought to be of particular importance in human speech processing. To investigate the function of these neurons, we used a mouse line that labels a subset of neurons in the small cell cap and performed patch-clamp electrophysiological recordings from labeled neurons.

Methods: Jing et. al (2025) used FISH to demonstrate that Penk was expressed in SCC neurons. Therefore, we crossed Penk-Ires-Cre mice with Ai14-TdTomato reporter mice to label a subset

of neurons in SCC. Next, we performed immunohistochemistry on Penk-Cre/TdTomato mice in brainstem slices to confirm that the expression was in the expected region of CN. For electrophysiology experiments, we collected sagittal brain slices containing SCC. We performed patch-clamp recordings from individually identified neurons in both current-clamp and voltage-clamp. Gap-free recordings were performed to collect spontaneous synaptic events. Series of either voltage steps or current steps were used to measure membrane and electrophysiological properties such as input resistance, capacitance, firing rate, and characterize voltage-gated currents.

Results: Recordings from Penk+ neurons in SCC indicate that these neurons have a high input resistance ($\sim 1.02 \pm 0.28$ G Ω), low capacitance (9.3 ± 2.1 pF), and are very excitable, consistently able to be excited with current injections as low as 5 pA. Voltage-clamp recordings revealed two different distinct types of outward currents, with one producing a flat, constant current ($n=4$) and the other displaying an inactivating current ($n=10$). All of these neurons displayed a voltage-dependent I_h current, with an activation voltage beginning at ~ -84 mV and becoming more prominent at more negative voltages.

Conclusions: Here, we used the Penk-Ires-Cre mouse line to target neurons in the small cell cap. Using patch-clamp electrophysiology, we demonstrate that these neurons have several unique properties, including a high input-resistance, a prominent I_h current, and a low rheobase. To understand the role of these neurons in auditory processing, further experiments manipulating their synaptic inputs and post-synaptic targets will be required.

M68. Modeling of Dorsal Cochlear Nucleus Reveals Connectivity Underlying Response Profiles

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Background: The dorsal cochlear nucleus (DCN) plays a critical role in auditory processing, including contributing to sound source localization, suppression of self-generated sounds, and transformation of complex spectral patterns (Oertel and Popper 2002, Young and Davis 2002, Singla et al. 2017). Data-driven computational modeling provides a powerful framework for investigating these functions, informing the understanding of experimental results and guiding future experimental studies.

Methods: We develop biophysically realistic cell and network models to investigate how connectivity within the DCN modulates representations of auditory stimuli. Using the CNModel platform (Manis and Campagnola 2018), we simulate a fusiform cell receiving synaptic input from excitatory auditory nerve fibers and inhibitory D-stellate and tuberculoventral cells. Auditory nerve inputs are driven by an auditory periphery model (Zilaney et al. 2014) that generates spike trains in response to pure tone stimuli.

Results: We explore the dynamical behaviors of a fusiform cell model by generating response maps that represent the evoked firing rate in response to model auditory stimuli of varying frequencies and sound levels. We demonstrate that distinct type III and IV response profiles emerge from different combinations of excitatory and inhibitory synaptic strengths.

Conclusions: These results highlight how subtle variations in network connectivity can shape neuronal response properties and provide a mechanistic framework for understanding functions of the DCN. Additionally, we model high frequency hearing loss by simulating hair cell impairment in the auditory periphery model and explore how altered connectivity patterns could contribute to the emergence of tinnitus.

M69. Open Board

M70. Contactin-2 (CNTN2) Expression in Developing Chick Inner Ear Neurons

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Background: During development of the inner ear, neuron progenitors extend projections from their soma centrally toward the brain and peripherally toward the inner ear organs. Neuronal projections rely on cues from their environment to navigate correctly to their targets. Cell adhesion molecules (CAMs) have been shown to play a role in axonal pathfinding processes, including an immunoglobulin super-family CAM, Contactin-2 (CNTN2).

Methods: To explore whether CNTN2 might play a role in axonal pathfinding of inner ear neurons, we characterized the spatiotemporal expression in chick auditory and vestibular neurons, by performing immunohistochemistry from E3-E7. CNTN2 expression was compared to the well-known neuronal label, TuJ1 as well as additional labels NCAM (Neuronal CAM), GATA3 (auditory neurons), and Myosin VIIa (vestibular neurons).

Results: CNTN2 was expressed in auditory and vestibular neuron soma and projections at all ages that were observed.

Conclusions: These data show that CNTN2 was present during the developmental stages when pathfinding is taking place, suggesting a role for CNTN2 in the pathfinding process of inner ear neurons.

M71. The Function of CaMKIV in Spiral Ganglion Neuron Development

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Background: Spiral ganglion neurons (SGNs) are the primary auditory neurons that form synaptic connections with hair cells in the cochlea and transmit auditory information to the brainstem. SGN-hair cell contacts are critical for hearing and are lost in various forms of hearing loss. In this research, we have focused our attention on activity-dependent aspects of SGN development, an area that is not well understood.

Ca²⁺/calmodulin-dependent protein kinase IV (CaMKIV) is crucial for aspects of neuron function, such as gene expression and neurotransmitter release, and is a target gene for autism spectrum disorders in humans. CaMKIV was shown by others to facilitate SGN survival and neuron branch dynamics in vitro. In previous studies (manuscript in preparation), we found that CaMKIV is a downstream effector of the purinergic receptor P2RX3: developing SGNs lacking

P2rx3 showed increases in SGN activity and levels of activated CaMKIV. In this present study, we addressed the hypothesis that CaMKIV is a downstream effector of SGN activity and that CaMKIV is an important regulator of SGN differentiation and survival.

Methods: We performed immunohistochemistry on P2rx3 knockout mice and cochlear cultures treated with MRS2500 to investigate CaMKIV levels in the context of high vs. low SGN activity. We also performed immunohistochemistry on CaMKIV knockout mice to characterize the role of CaMKIV in SGN subtype differentiation and its interaction with pCREB in the cochlea at different developmental stages.

Results: CaMKIV protein levels were increased in the nuclei of the SGNs from mice lacking P2RX3 (P2rx3 knockout mice). The increased CaMKIV levels coincided with increased pCREB activation, SGN branch arborization, and a brief increase in spontaneous activity. Our data also show a decrease in CaMKIV protein levels in samples treated with MRS2500 (low activity model). Collectively, these data suggest CaMKIV responds to changes in SGN synaptic activity. In addition, we observed decreased pCREB levels in neonatal mice lacking CaMKIV (camk4 knockout mice), suggesting a direct relationship between CaMKIV and pCREB. Furthermore, adult mice lacking CaMKIV showed an increase in the number of 1c SGNs and an increase in pCREB levels, indicating a role for CaMKIV in SGN differentiation and pCREB regulation.

Conclusions: CaMKIV expression is dependent on activity in the cochlea. Our data indicate a relationship between CaMKIV and pCREB in the cochlea during development. Our data also indicates that CaMKIV plays a role in SGN differentiation. These data provide insight into activity-dependent mechanisms associated with SGN development. In current research, we are investigating other functions of CaMKIV in SGNs during development, like branch refinement and survival.

M72. From Alzheimer's Disease to Hearing Loss: The Role of APOE in SGN-Hair Cell Connectivity

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Background: Hearing loss and Alzheimer's Disease prevalence in the US is astounding, with each affecting over 10% of the population. Recently "sensorineural hearing loss" (the most common form of hearing loss) was found to be a risk factor for Alzheimer's Disease.

Sensorineural hearing loss involves poor survival of both hair cells and SGNs, but how it contributes to Alzheimer's Disease is not understood. In this project, we will close this gap by focusing on Apolipoprotein E (APOE), a protein commonly associated with Alzheimer's Disease. Previous research has shown that APOE is necessary for maintaining normal hearing acuity, suggesting it may have protective role in the auditory system. We hypothesize that APOE is required to maintain SGN survival, and that disruption of APOE increases vulnerability to both developmental degeneration and noise-induced cochlear damage.

Methods: Using littermate controlled APOE knockout and wild-type mice, we combined immunohistochemistry, cochlear explant, confocal microscopy, and quantitative image analysis to examine SGN survival, cochlear structure, and macrophage activation on developing cochlea.

Results: Our preliminary data show that loss of Apoe reduces SGN density within Rosenthal's canal, as quantified by neuronal counts, consistent with impaired SGN survival. We also observed increased expression of the neuronal stress marker S100B within the spiral ganglion, suggesting that Apoe deficiency enhances stress responses in cochlear neurons. In parallel, immunostaining revealed heightened macrophage activity, with cells exhibiting enlarged soma and reduced branching complexity, indicative of an activated morphology. Together, these findings suggest that Apoe deficiency not only compromises SGN survival but also promotes both neuronal stress and dysregulated immune activation in the cochlea.

Conclusions: Our results support a protective role for APOE in maintaining SGNs, mitigating cochlear damage. These findings provide new insight into how APOE may connect auditory dysfunction with Alzheimer's disease pathology.

M73. Examination of Amyloid Precursor Protein's Role in the Development of the Utricle

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Background: The amyloid precursor protein (APP) is a membrane bound protein which, under normal conditions, plays important roles in brain development, such as neurogenesis, neuronal maturation, and axon guidance. APP is first cleaved by either alpha or beta secretase, then subsequently cleaved by gamma secretase. Both of these processing pathways create protein fragments that can influence cell function, such as amyloid beta, which is associated with Alzheimer's disease (AD). Another fragment made by both of these processing pathways is the amyloid intracellular domain (AICD). Literature suggests that AICD is degraded when made by the alpha secretase pathway. However, when made by the beta secretase pathway, AICD acts as a transcription factor, regulating expression of genes important for development and cell survival. The APPNL-F/NL-F mouse is a knock-in line where APP is altered to incorporate two mutations found in human AD patients to favor beta secretase cleavage of APP. Thus, amyloid beta, and possibly AICD, are increased without overexpression of APP itself. Our preliminary results show morphological differences in the utricles of APPNL-F/NL-F mice compared to C57Bl/6J mice at 2 months of age. Since previous studies have shown that AICD can repress expression of Sox2, and Sox2 plays an important role in the development of the vestibular system, we hypothesize that an increase in production of AICD occurs in APPNL-F/NL-F mice, which alters development of the utricle.

Methods: Temporal bones were collected from male and female APPNL-F/NL-F and C57Bl/6J mice at 2 months of age. Immunofluorescent staining was performed using the hair cell marker, myosin VIIa, with type II hair cells and supporting cells identified by Sox2 labeling. Stereocilia bundles and phagosomes were labeled using phalloidin, and oncomodulin was used to identify and measure the striolar area.

Results: Comparison of 2 month old APPNL-F/NL-F utricles to C57Bl/6J utricles yielded several morphological differences. While there were no differences in the number of type I or type II hair cells, stereocilia bundles or the total area of the sensory epithelia, APPNL-F/NL-F utricles had a smaller striolar area than C57Bl/6J utricles. We also found a decreased number of phagosomes in APPNL-F/NL-F utricles, as well as the presence of extracellular spaces, or voids,

around many type I hair cells. Based on these findings, we are currently examining neonatal APPNL-F/NL-F utricles to assess if there are deficits in hair cell differentiation and maturation, or stereocilia bundle and innervation development.

Conclusions: Morphological differences seen in 2 month old APPNL-F/NL-F utricles when compared to C57Bl/6J utricles are not easily explained and may suggest either developmental deficits or toxicity caused by increased amyloid beta

M74. Essential Functions of the PDZ Domain Scaffold Protein Afadin in PCP Regulation in the Mouse Cochlea

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Background: The planar cell polarity (PCP) pathway aligns cell polarity along the tissue plane and is required for a myriad of developmental processes in vertebrates, including convergent extension and neural tube closure. In the cochlea, the PCP pathway regulates the uniform orientation of the stereociliary hair bundles and plays a non-autonomous role in cochlear supporting cells to guide the peripheral axons of type II spiral ganglion neurons (SGNs) (Ghimire et al., 2018). Accumulating evidence suggests that PCP signaling at adherens junctions of epithelial cells is influenced by junctional actomyosin contractility (Clancy et al., 2025). However, the interplay between actomyosin contractility and PCP signaling in the developing cochlea is poorly understood. Afadin is a PDZ-domain scaffolding protein that links cell adhesion proteins such as E-cadherin and Nectins to the actin cytoskeleton. Its expression and junctional recruitment increase in response to elevated junctional actomyosin contractility, thereby stabilizing cell-cell adhesion under mechanical load (Choi et al., 2016). Therefore, we hypothesized that Afadin regulates PCP signaling in the cochlea by coordinating actomyosin contractility and asymmetric localization of core PCP proteins. To test this, we have generated and analyzed PCP defects in afadin conditional knockout (cKO) mice.

Methods: To determine the role of afadin in cochlear development, we generated Afadin^{cKO} mice using two Cre drivers that target different stages and cell populations in the developing cochlea: pan-inner ear deletion of afadin using Foxg1cre (Afadin^{ΔIE}), and deletion of afadin in hair cells and supporting cells in the organ of Corti using Fgf20cre (Afadin^{ΔOC}). Cochleae from Afadin^{cKO} and littermate controls were dissected at E18.5 for Afadin^{ΔIE}, or at P0 for Afadin^{ΔOC}. Samples were immunostained with acetylated tubulin, phalloidin, and α-catenin, to label the kinocilium and SGN neurites, hair bundles and cortical actin belts, and cell-cell junctions, respectively. Defects in cellular patterning, kinocilium positioning, hair bundle orientation and SGN peripheral axon guidance were quantified using published protocols.

Results: Preliminary results show that both Afadin^{ΔIE} and Afadin^{ΔOC} displayed PCP defects, including kinocilium mispositioning, hair bundle misorientation, and type II SGN axon turning errors. This indicates the requirement of Afadin in regulating PCP in the cochlear epithelium. Compared to Afadin^{ΔOC} mutants, Afadin^{ΔIE} displayed more severe PCP defects, such as fragmented hair bundles and impaired cellular patterning in the OC, including disrupted cellular mosaic, irregular cell circumferences, the formation of hair cell clusters separated by gaps devoid of hair cells. To understand the molecular basis for the PCP defects, we are currently

investigating how afadin loss affects the subcellular localization of core PCP proteins and cell adhesion molecules.

Conclusions: The loss of function mutant phenotypes indicate that Afadin is required for PCP regulation in the cochlear epithelium, and likely plays a non-autonomous role in guiding the type II SGN peripheral axons.

M75. A Single-Nucleus Resolution Transcriptomic Atlas of the Mouse Utricle Across Maturation

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Background: The inner ear goes through a complex process of maturation from the embryo to adulthood. These steps set up the inner ear's sensory organs which rely on mechanosensory hair cells (HCs) for the detection of sound and acceleration. These cells are sensitive to input and can be damaged by ageing, disease or, in the case of auditory HCs, by loud noise. In adult mammals, sound-sending HCs in the cochlea are not regenerated, however, acceleration-sensing HCs in the utricle have a limited capacity for regeneration during postnatal stages in mouse, that tapers to marginal HC production in adulthood.

As such, the utricle represents a potential regenerative niche in adult mammals, but knowledge of how this tissue matures at key developmental timepoints is understudied. During maturation, gene expression changes across multiple cell types in concert to result in a functioning inner ear. Although we are aware of key genes involved in HC regeneration, the transcriptomic identity of cells within the utricle has not yet been described at high temporal resolution across maturation. We therefore lack an understanding of how maturation impact HC regeneration potential.

Methods: Whole utricles were obtained from mice at seventeen timepoints across the first five months of life, between E14 and P147. These utricles were used to generate libraries for single nucleus RNA sequencing using combinatorial barcoding.

After sequencing, read mapping, quantification and quality control was performed to remove doublets and low-quality nuclei and produce the final dataset. Non-linear dimensionality reduction was used as a basis for Leiden clustering, with cluster stability analysis, to identify stable clusters of cells across the dataset.

Further analysis of cell and transcriptomic identity was performed using known markers from the literature alongside statistical analyses.

Results: A transcriptomic dataset of 120 000 nuclei was produced. This atlas includes key cell types within the sensory epithelia including supporting cells, hair cells, and mesenchyme, as well as rarer and lesser described cells such as melanocytes, macrophages, and pericytes.

Gene expression programmes associated with maturation were identified using differential gene expression analysis and consensus non-negative matrix factorisation. Using this approach, we identified cell types, subtypes, and states throughout the utricle and described transcriptional dynamics of utricular hair cell maturation.

Conclusions: This novel transcriptomic atlas provides a resource for investigating the maturation of the mouse utricle at high temporal resolution, covering key developmental milestones such as the onset of vestibular function at P4. We are actively working towards sharing the data through gEAR to support ongoing research efforts in the field.

M76. Neural Crest–Derived Melanocytes in the Mammalian Stria Vascularis: Evolutionary and Developmental Insights From Mouse–Chick Comparison

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Background: Several decades ago, Petrus Waardenburg identified a strong association between deafness and hypopigmentation in human patients. It is now well established that in the mammalian cochlear stria vascularis (SV), the intermediate cells are melanocytes derived from the neural crest. Mutations in genes affecting neural crest or melanocyte development are frequently linked to hearing impairment. In adult mammals, the SV comprises three distinct cellular layers, each originating from different embryonic tissues. The innermost layer, adjacent to the scala media, contains marginal cells derived from cochlear epithelial cells. The outer layer consists of basal cells, which differentiate from surrounding cochlear mesenchymal cells. The intermediate layer, populated by melanocytes, expresses *Kcnj10*, which encodes the inwardly rectifying K⁺ channel Kir4.1, essential for generating the endocochlear potential (EP, +80 mV). In contrast, *Kcnj10* is not expressed in the chick cochlea, and the EP in chicks is considerably lower (+15 mV). These observations led us to hypothesize that melanocytic intermediate cells represent a novel neural crest–derived contribution in mammals, contributing to the high EP compared with avian species.

Methods: Postnatal and embryonic mice, along with embryonic chicks, were analyzed using immunostaining and hybridization chain reaction (HCR). Unilateral homotopic neural crest (NC) grafts were performed in chick at the 8-somite stage (HH9+), from a transgenic GFP donor to a WT host. Pre-migratory NC was surgically excised from the dorsal neural tube of the donor and grafted to the corresponding position in the host (corresponding host NC is excised and removed). Grafted embryos were then cultured in ovo for 7.5 days at 38°C, until ~HH35.

Results: *Wnt1*Cre lineage tracing in mice revealed that neural crest–derived melanocytes in the cochlear SV express high levels of *Kcnj10* and other melanocyte-specific genes. Neural crest cells contribute to both the glial cells of the ganglia and the intermediate cells of the SV in the mammalian cochlea. In contrast, no melanocytes are observed in the developing chick cochlea, and grafting experiments indicate that neural crest cells in chicks contribute exclusively to glial cells. *Mitf*⁺ melanocytes colonize the mouse otic vesicle but are absent in the chick otic vesicle during early development. Notably, a cluster of *Mitf*⁺ melanocytes originates from Schwann cell precursors along cranial ganglia IX/X, which subsequently migrate to populate the otic vesicle in mouse embryos. By contrast, melanocyte differentiation is not detected in the cranial ganglia IX/X of chick embryos at equivalent stages, suggesting an evolutionary acquisition in mammals that confers melanocyte differentiation potential to these glial cells.

Conclusions: Schwann cell precursors along cranial ganglia IX/X posterior to the otic vesicle evolved the ability to generate melanocytes, contributing to the formation of the intermediate cells in the SV that underlie the elevated EP in mammals.

M77. Progress in the Construction of Inner Ear Organoids

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Background: The study of the human inner ear and its sensory hair cells has long been hampered by the extreme scarcity of native tissue for research. This scarcity creates a significant barrier to understanding inner ear physiology and developing treatments for hearing and balance disorders. Organoids, which are self-organizing three-dimensional structures derived from stem cells, present a promising in vitro solution to model these scarce tissues. The development of advanced 3D culture systems is critical for this endeavor, as they provide the necessary environment to not only guide stem cell proliferation and differentiation but also to promote the formation of complex, higher-order structures that mimic native organs.

Methods: Our methodological approach involved the design, fabrication, and validation of several bioengineered systems. A novel conductive hydrogel was synthesized by incorporating a conductive polymer network into a biocompatible hydrogel matrix. This substrate was characterized for its electrical and mechanical properties before being used for organoid culture. Inner ear organoids were generated from human pluripotent stem cells using a established stepwise differentiation protocol, which was adapted for culture on the novel hydrogel substrate.

For the chip-based models, we utilized soft lithography to fabricate polydimethylsiloxane (PDMS) microfluidic devices. The cochlea-on-a-chip was designed with a central culture chamber for housing individual organoids, connected to microchannels for the controlled perfusion of culture media and test compounds.

The blood-labyrinth barrier (BLB)-on-a-chip model was designed to comprise two parallel microchannels separated by a porous membrane. Endothelial cells were cultured on one side of the membrane to form a vascular channel, while pericyte cells and inner ear organoid-derived cells were cultured on the opposite side to recreate the BLB interface. Functional validation of the models included immunofluorescence staining and confocal microscopy for key cell markers (e.g., MYO7A for hair cells), quantitative PCR for gene expression analysis, and measurements of transepithelial electrical resistance (TEER) to assess BLB integrity. Drug permeability across the BLB model was quantified using liquid chromatography-mass spectrometry (LC-MS).

Results: The use of the custom conductive hydrogel significantly enhanced the differentiation and maturation of sensory hair cells within the inner ear organoids. The cochlea-on-a-chip platform was successfully implemented for the robust culture of cochlear organoids and proved to be a viable system for high-throughput drug screening. Finally, the inner ear-on-a-chip model effectively mimicked the natural BLB, providing a functional tool to assess the permeability of various therapeutic compounds across this critical barrier.

Conclusions: In summary, this body of work demonstrates significant progress in the development of sophisticated in vitro models for inner ear research. The constructed inner ear organoids and organ-on-a-chip platforms aim to serve as reliable tools for future physiological and pathological studies, offering new avenues for drug discovery and toxicity testing.

M78. Stk32a Acts to Define Directional Properties of Zebrafish Hair Cells

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Background: In vertebrate otolith organs and in the zebrafish posterior lateral line (pLL), hair bundles are organized into opposing populations to enable bidirectional detection of stimuli. In the pLL, hair cells with opposite orientations exhibit distinct mechanosensitive properties, and afferent neurons selectively innervate hair cells of the same orientation to transmit directional information centrally. Previous work in the pLL identified the transcription factor *Emx2* as a key regulator of hair bundle orientation, mechanosensory properties, and afferent selectivity. More recently, studies in mouse have shown that the kinase *Stk32a* is transcriptionally repressed by *Emx2* to govern hair bundle orientation. However, whether *Stk32a* plays a functionally conserved role in hair bundle orientation in zebrafish, and whether it influences mechanosensory properties or afferent selectivity, remains unknown.

Methods: For our study, we analyzed *stk32a* zebrafish mutants. We performed immunostaining to visualize hair bundle orientations in the zebrafish inner ear and pLL. Using transgenic lines, we conducted GCaMP6s-based measurements to assess mechanosensitive responses in pLL hair bundles. Finally, we employed synaptic immunostaining and sparse labeling approaches to visualize ribbon synapses and to examine the orientation selectivity of afferent neurons, respectively.

Results: We find that in the zebrafish pLL, *stk32a* mutants exhibit hair bundle orientations opposite to those of *emx2* mutants. Whereas *emx2* mutants orient all bundles to respond to anterior flow, the majority of bundles in *stk32a* mutants are oriented to sense posterior flow. In the cristae of *STK32a*-deficient zebrafish, hair cell orientations are slightly off-axis, while orientations in the otolith organs remain largely unaffected at larval stages. Calcium imaging in the pLL further revealed that posterior-sensitive hair cells in *stk32a* mutants display reduced mechanosensitive responses—opposite to the enhanced responses of anterior-sensitive hair cells observed in *emx2* mutants. Our preliminary synaptic immunostaining and sparse labeling indicate that *Stk32a* is not required for afferent selectivity.

Conclusions: Our findings demonstrate that *Stk32a* plays a conserved role in establishing hair bundle orientation in zebrafish, with *stk32a* mutants conferring orientation phenotypes opposite of *emx2* mutants. Beyond orientation, *Stk32a* influences the mechanosensory properties of hair cells but appears dispensable for afferent selectivity. Together, these results identify *Stk32a* as a critical regulator of hair cell orientation and mechanosensitivity, providing new insights into the transcriptional pathways that control directional sensitivity in vertebrate sensory systems.

M79. Cryo-Em Structures of Prestin Reveal Compact and Asymmetric States With Ion Binding Similar to Pendrin

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Background: Outer Hair Cells (OHCs), through their electromotile properties, are responsible for cochlear amplification. Prestin, the motor protein located in the lateral membrane of OHCs, undergoes conformational transitions distinct from canonical SLC26 transporters. Previous cryo-EM studies identified prestin in expanded and contracted states, but the mechanisms of ion binding and conformational asymmetry remain unresolved.

Methods: Purified prestin in the presence of 200 mM NaCl-/40mM NaSCN was applied to Quantifoil R1.2/1.3 300 Au mesh grids and vitrified in liquid ethane. Cryo-EM data were collected on a Titan Krios G4 at 300 kV with a K3 detector. Over 15,000 dose-fractionated movies were recorded with defocus values ranging from -2.0 to -2.5 μm . Data was processed in CryoSPARC, and models were refined using COOT and PHENIX.

Results: We determined multiple prestin structures at 3.1–3.9 Å resolution in the presence of SCN^-/Cl^- , corresponding to canonical, compact and asymmetrical states. Ion densities consistent with chloride and thiocyanate (SCN^-) were resolved at both internal and external binding sites. SCN^- interacts with serine, lysine and phenylalanine residues, forming rod-like densities. A striking asymmetry was observed between protomers: one protomer adopts a compact conformation with TM6–TM7 and the extracellular loop (ECL3) opened, exposing an external ion-binding site corresponding to the equivalent extracellular site in pendrin, an anion transporter in the SLC26 family, whereas the other protomer retains a canonical closed-loop state without external ion binding. In the compact protomer, inward movement of TM1 is coupled to conformational displacement of TM10, reflecting coordinated helical rearrangement.

Conclusions: Our findings reveal that prestin dimers can adopt asymmetric conformations with ion binding to an extracellular site, loop gating, and TM1/TM10 movements. These compact and asymmetric states provide new mechanistic insights into prestin's voltage-sensitive transitions and electromotility.

M80. Conformational Changes Underlying Electromechanical Transduction in Prestin Resemble a Partial Transport Transition in Pendrin

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Background: Prestin (SLC26A5) is a member of the SLC26 anion transporter family. It diverged as the voltage-driven motor of cochlear outer hair cells, where its conformational changes underlie electromotility and cochlear amplification. We previously resolved a 3.6 Å cryo-EM structure consistent with the contracted state, and more recently a 3.24 Å cryo-EM structure with an external anion-binding in the extracellular region. Prestin transitions between compact (CS) and extended (ES) conformations in a voltage-dependent manner, with depolarization favoring CS and hyperpolarization favoring ES. As the expanded state remains structurally undefined, we employed molecular dynamics (MD) simulations to model the transition from the contracted to expanded state.

Methods: We performed multi-microsecond molecular dynamics simulations of prestin starting from the compact state (PDB: 7SUN) in the presence of chloride using GROMACS and ANTON

simulation. These trajectories were compared with known inward (PDB: 7WK1) and outward (PDB: 7WLE) facing structures of pendrin (SLC26A4), another SLC26 family member. In parallel, electrophysiological experiments were performed on pendrin to assess nonlinear capacitance and voltage-dependent conformational changes.

Results: While previous structural models of the ES conformation relied on ligand stabilization, we used multi-microsecond molecular dynamics simulations starting from the CS state in the presence of chloride to generate an ES model. This model resembles the inward-facing (IF) conformation of pendrin (SLC26A4), another SLC26 family member whose structures in both IF and outward-facing (OF) states are known. Prestin, however, undergoes only a partial OF-to-IF-like transition. Simulations also reveal a previously uncharacterized compact conformation that resembles OF pendrin, and an extracellular chloride-binding site analogous to one in pendrin. Importantly, the external anion-binding site predicted by MD simulations was confirmed in the 3.24 Å cryo-EM structure of prestin. Electrophysiological experiments confirm that pendrin exhibits non-linear capacitance, similar to prestin, indicating that pendrin undergoes conformational changes in a voltage-dependent manner. Taken together, these findings suggest that prestin and pendrin share core structural properties, including area expansion and voltage dependency, which together form the basis of the area motor model of electromechanical transduction.

Conclusions: Our combined simulations, cryo-EM and electrophysiology reveal that prestin undergoes partial transport-like transitions that resemble those of pendrin but appear to be adapted for voltage-dependent electromechanical function. These results suggest that prestin and pendrin share fundamental structural principles, but prestin's unique role in cochlear amplification arises from its specialized cellular context, perhaps including dense packing in the lateral membrane of outer hair cells.

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M81. Unraveling the Role of Presynaptic Autophagy in Inner Hair Cell Ribbon Synapse Development

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Background: In the mammalian cochlea, auditory inner hair cells (IHCs) convert acoustic stimuli into a chemical signal to activate spiral ganglion neurons (SGNs). The IHCs connect to the afferent SGN terminal via specialized ribbon synapses, which are optimized for the indefatigable and temporally accurate release of glutamate, and thus continuous signal transduction. The resulting extraordinarily high metabolic rate of the IHC synapse hence poses a challenge to the maintenance of presynaptic homeostasis. At conventional neuronal synapses, this balance is maintained by presynaptic autophagy: a tightly regulated degradation process by which excess or dysfunctional proteins and organelles are cleared from the axonal terminal. A similar involvement of autophagy in IHCs appears likely, especially since autophagic disruptions have been linked to sensorineural hearing loss. Moreover, the recently reported upregulation of autophagy during early postnatal development suggests a role for presynaptic autophagy in

maturation refinement of the ribbon synapse. However, the exact presynaptic autophagy pathway in IHCs has remained largely elusive thus far.

Methods: In this project, we combine organotypic organ of Corti cultures with various live-cell imaging techniques, super-resolution and electron microscopy. Our comprehensive approach facilitates the mapping of the presynaptic autophagic flux in developing IHCs, as well as the tracing of autophagosome dynamics in real time. Furthermore, we assess the correlation between ribbon synapse maturation, synaptic activity, and autophagic degradation using pharmacological and optogenetic tools.

Results: We found that spherical membranous compartments localized intracellularly within the IHC, and were most prominent at the presynaptic active zone (AZ). The majority of these structures presented with highly dynamic ribbon precursors engaged with their outer membrane. Moreover, the structures displayed bouts of rapid mobility, as well as colocalization with autophagic markers and, in some cases, association with lysosomes – all classical hallmarks of autophagosomes. Interestingly, we also observed the boutons of innervating afferent SGNs to frequently appear encapsulated by the IHC membrane, creating a spherical postsynaptic space protruding into the IHC cytosol. Partnering the enveloped postsynapse, ribbon precursors localized in a circular configuration typical for developing IHC presynaptic AZs. This structural differentiation might thus present an early stage of the membranous autophagosomes located intracellularly, and thereby represent part of the IHC autophagic pathway.

Conclusions: Our findings suggest that autophagy is highly prevalent at the presynaptic AZ of IHCs during postnatal development and may follow an intricate and possibly unique pathway. Especially during the synaptic pruning phase – in which surplus synapses are eliminated in the runup to hearing onset – autophagy may play a fundamental role in the redistribution and degradation of ribbon synapse material and thereby facilitate the adequate maturation of auditory ribbon synapses.

M82. A Survey of Class 1 Myosins in Hair Cells and Their Impacts on Hearing

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Background: Sensory hair cells (HCs) harbor specialized apical microvilli called stereocilia that are deflected in response to sound stimuli. Stereocilia are composed of a dense core of bundled actin and have mechanically-gated ion channels in their membranes that mediate hair cell mechanotransduction (MET). Class 1 myosins are a family of unconventional myosins that bind to both actin and the plasma membrane. Among them, Myo1C has been implicated in hair cell MET, but its role remains debated. Recent RNA sequencing data suggest several class 1 myosins are present in the inner ear, but relatively little is known about their specific expression and function. We hypothesize that class 1 myosins are involved in linking the stereocilia actin core to the cell membrane and thus are important for MET and hearing function.

Methods: To overcome well-documented limitations of antibody specificity, we used CRISPR/Cas9 to generate knock-in (KI) mouse lines with an HA tag inserted into Myo1C,

Myo1D, Myo1E, and Myo1H. HA tag imaging enabled subcellular localization studies. We are performing immunoprecipitation of Myo1s and will subsequently use mass spectrometry to identify binding partners. To assess function, we created knockout (KO) mice for Myo1D, Myo1E, and Myo1H.

Results: Myo1C was expressed at low levels in vestibular hair cells but absent in auditory hair cells. Myo1E was not observed in neither vestibular nor cochlear hair cell stereocilia. In contrast, Myo1D and Myo1H showed prominent stereocilia localization, displaying cell-type and intra-stereocilia differences. Myo1H was initially expressed in all hair cells, but during postnatal development became highly restricted to the stereocilia of vestibular and outer hair cells. Myo1D could be found in the stereocilia of both IHCs and OHCs. Assessment of hearing in the single and double KO mice are in progress.

Conclusions: By analyzing publicly available gene expression data, we identified and determined the localization pattern of the main class 1 myosins expressed in the inner ear. We found that Myo1D and Myo1H are both present specifically in stereocilia. Myo1C was localized in vestibular hair cell stereocilia at low levels, and absent in auditory hair cells, while Myo1E was absent from stereocilia. Loss-of-function studies of Myo1D and Myo1H will clarify their roles in MET and address a long-standing debate regarding class I myosins in hair cell function

M83. Influence of Myosin Regulatory Light Chain and Myosin Light Chain Kinase on the Physiological Function of Inner Ear Hair Cells

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Background: In the receptor organs of the inner ear, hair cells (HCs) detect mechanical stimuli such as sounds and accelerations by deflection of their hair bundles. Applied mechanical stimuli are very small; therefore, HCs have “active process” to amplify the signal. The tension of tip link is thought to be important for active process. Myosin regulatory light chain (RLC) and non-muscle myosin II (NM2) are expressed at the apical surfaces of hair cells, and NM2 and the phosphorylation of RLC by myosin light chain kinase (MLCK) have earlier been shown to regulate the shapes of hair cells’ apical surfaces in rodents. Furthermore, unconventional myosins expressed at the tip of stereocilia are believed to maintain the tension of tip link. The aim of our study was to elucidate the function of myosin molecules on hair cell physiology and active process.

Methods: We investigated the expression of NM2 and RLC in the bullfrog’s saccule by immunostaining. Using NM2 and MLCK inhibitors, we measured physiological parameters essential for the active process such as stiffness and spontaneous oscillation of hair bundles, and resting open probability of mechanotransduction channel. We constructed a two-compartment experimental chamber which reproduced physiological conditions; the upper chamber contained endolymph, and the lower chamber contained perilymph. In addition, we recorded auditory brainstem responses in ten mice after transtympanic injection of an MLCK inhibitor.

Results: We confirmed the expression of NM2A/B and MYL9 on the apical surfaces of hair cells and of NM2A and MYL12A in hair bundles. We found that NM2 and MLCK inhibitors reduce the stiffness of hair bundles from the bullfrog's saccule. Moreover, MLCK inhibition inhibits the spontaneous oscillation of hair bundles and increases the resting open probability of transduction channels. In both experiments, recovery effects were observed after washout of the inhibitors. In contrast, NM2 inhibitor did not show any changes in spontaneous oscillation and resting open probability. In addition, MLCK inhibition elevates hearing thresholds in mice.

Conclusions: We conclude that NM2 and the phosphorylation of RLC modulate the physiological function of hair cells. In particular, RLC phosphorylation is essential for the regulation of tip-link tension, thereby contributing to the maintenance of the normal operating conditions of hair bundles. Myosin-dependent regulation is a key component of the active process in HCs. Our results suggest that myosin activity is also important for auditory function, and a potential therapeutic target of hearing loss.

M84. Isoform-Specific Functions of Espin Variants in Auditory and Vestibular Hair Cells

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Background: The growth, turnover, and packing of the stereocilia f-actin core are governed by actin-binding proteins, among which the crosslinker Espin is particularly consequential. Espin's four isoforms (1–4), arising from alternative start sites, are exceptionally potent at building parallel actin bundles. Previous work on the jerker mouse has demonstrated that loss of Espin causes malformed, unstable stereocilia and severe congenital deafness with vestibular deficits in mammals. Prior data from our lab suggest Espin may also participate in actin core repair—colocalizing with XIRP2 at stereocilia lesions—yet Espin's exact role in repair remains undefined. Furthermore, despite isoform-specific biochemical differences, most in-vivo work has emphasized Espin-1, leaving how individual isoforms support stereocilia development, maintenance, and repair across and within hair-cells largely unknown.

In the present study, we begin to resolve isoform-specific contributions of Espin—especially Espin-4—to stereocilia development, maintenance, and repair across auditory and vestibular hair cells. Using mice with specific deletions in Espin 1-3 or Espin 4, we will test Espin-4 sufficiency and necessity in auditory and vestibular function and probe Espin's involvement in actin repair at stereocilia lesions.

Methods: We generated an Espin 1-3 specific KO line (Espin Δ 1–3) that only expresses Espin 4, confirmed complete deafness by ABR, and examined cochlear stereocilia by immunofluorescence to assess bundle morphology and protein distribution in development and adulthood. Future work will incorporate Espin 4 specific KO (Espin Δ 4) and HA tagged (HA-Espin4) mice to test necessity and resolve isoform-specific localization within stereocilia, alongside live Ca²⁺ imaging during fluid-jet deflection and SEM ultrastructural assays.

Results: Espin Δ 1–3 mice exhibit complete deafness with no overt vestibular dysfunction, diverging from the phenotypic vestibular defects seen in pan-Espin KO jerker mice. Cochlear hair-bundle defects appear more impactful than vestibular defects, indicating differential isoform requirements between systems. Interestingly, unlike the jerker mouse in which pan-espin KO

leads to complete degradation of all cochlear stereocilia, *Espin* Δ 1–3 mice show robust 1st row stereocilia morphology well into adulthood. These data motivate two planned tests: (i) sufficiency—whether *Espin*-4 alone can sustain stereocilia morphology and mechanotransduction, particularly in vestibular hair cells; and (ii) necessity—whether isoform specific loss of *Espin*-4 (*Espin* Δ 4) exacerbates structural/functional defects beyond those seen in *Espin* Δ 1–3. Ongoing work applies SEM/IF, calcium imaging, ABR, and VOR across genotypes to resolve isoform-specific contributions.

Conclusions: Preliminary findings support a model in which *Espin* isoforms differentially support stereocilia integrity across auditory and vestibular hair cells and across rows of stereocilia within auditory hair bundles. Systematic testing of *Espin*-4 sufficiency and necessity will clarify isoform-level mechanisms of stereocilia development, maintenance, and repair, with implications for the pathogenesis of congenital, progressive, and noise-induced hearing loss.

M85. Molecular and Structural Basis for Limited Motility in Mouse Vestibular Kinocilia

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Background: Kinocilia are microtubule-based organelles present on the apical surface of hair cells in the inner ear. Although typically considered non-motile, recent observations suggest that vestibular kinocilia exhibit limited spontaneous motion in acute mouse semicircular canal preparations. This raises the possibility that they retain molecular and structural components of axonemal containing motile cilia. Outer dynein arms (ODAs), essential motor complexes in actively motile axonemes, have not been structurally characterized in vestibular kinocilia, leaving open the question of whether they contribute to force generation and/or play alternative mechanical roles. Moreover, the molecular basis for central pair apparatus in vestibular kinocilia has not been directly resolved.

Methods: We investigated vestibular kinocilia in situ using cryo-electron tomography (cryo-ET) of intact adult mouse preparations. Analyses focused on the presence, spacing, and organization of ODAs along the A-tubule of doublets. Tilt series were reconstructed, aligned, and examined for periodic densities consistent with dynein arm placement. In parallel, transcriptomic profiles (RNA-seq) from adult vestibular hair cells were analyzed to assess expression of axonemal genes. Expressed gene sets were mapped in silico onto high-resolution atomic models of the 96-nm repeat from human respiratory cilia (PDB: 8J07) and bovine sperm flagella (PDB: 9FQR). This integrative approach generated predicted composite models of vestibular kinocilia, including assessment of central pair components

Results: We identified ODA consistently positioned along the A-tubules of vestibular kinocilia, arranged at a regular 24-nanometer periodicity. This spacing matches the hallmark organization observed in motile cilia from trachea, sperm, and protists. Previously, our transcriptomic modeling focused on the 96-nm axonemal repeat associated with doublet microtubules, but this framework did not include the central pair complex (CPC), which lies within the axonemal lumen and follows a distinct periodicity. In the present study, RNA-seq analysis revealed transcripts for the majority of CPC-associated genes, supporting the prediction that vestibular kinocilia retain a central pair apparatus. Our updated models suggest that the internal C2 tubule

is well conserved, whereas some components specific to the C1 tubule are missing, consistent with a structurally asymmetric CPC.

Conclusions: This study provides the first structural evidence that mouse vestibular kinocilia contain outer dynein arms arranged with 24-nm spacing and based on predictive modeling, retain a central pair apparatus. Together, these canonical features of motile axonemes suggest that vestibular kinocilia preserve an architectural framework consistent with force generation or motility. While sub-tomogram averaging is still needed to resolve dynein conformational states and central pair density, our integrated cryo-ET and transcriptomic analyses support the view that vestibular kinocilia maintain key motile machinery elements. These findings lend structural and molecular support to reports of limited kinociliary motion and suggest that ODAs and CPC structures may contribute to subtle mechanical responses or dynamic regulation within the sensory epithelium.

M86. Investigating Direct Protein-Protein Interactions in the Inner Ear Mechanoelectrical Transduction Complex

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Background: The conversion of mechanical stimuli from sound waves to electrical signals interpreted by the brain is facilitated by the mechanoelectrical transduction (MET) channel complex of the sensory hair cells in the inner ear. Proteins transmembrane inner ear (TMIE) and calcium and integrin binding (CIB) are accessory subunits to the pore-forming transmembrane-channel-like proteins 1 and 2 (TMC1 and TMC2). The role of TMIE and CIBs in MET function is still unknown, and information on the interactions between these proteins is incomplete.

Methods: Here, we express and purify the cytoplasmic domains of murine TMCs, TMIE, and CIBs. Direct protein-protein interactions are measured using bio-layer interferometry.

Results: Our preliminary data indicate that the TMC1 N-terminal domain interacts with the cytoplasmic domain of TMIE less efficiently than the TMC2 N-terminal domain. This was later quantified through affinity calculations. Competition experiments showed a potential competitive interaction between TMCs, TMIE, and CIBs.

Conclusions: Our work provides information on the dynamics of MET complex regions that are not well-resolved by cryo-EM or other structural methods. This data is foundational for understanding the mechanism of deafness-causing mutations in these proteins, as well as MET complex formation in development.

M87. Noncanonical PAX6 Localization in Eurycea Salamanders and Zebrafish Neuromasts

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Background: PAX6 is a well-known transcription factor involved in the development of the eye, the central nervous system, and endocrine glands. Mutations in the gene encoding PAX6 cause human disorders such as aniridia. Building on our recent report of PAX6 immunolabeling in the apical appendages of hair cells in mechanosensory neuromasts of Eurycea salamanders, we hypothesized that PAX6 may associate with the actin cytoskeleton in the stereociliary bundle, given its expression pattern in this region. Neuromast organs in the lateral line of fish and amphibians provide excellent models for studying hair cells in their native environment, thanks to their accessibility. To enable future corroborative and functional assays interrogating PAX6's role at a noncanonical neuromast site, we next asked whether PAX6 is present in a more tractable model species, namely zebrafish (*Danio rerio*).

Methods: As a preliminary assessment, we performed immunohistochemical colocalization analysis in two Eurycea species (*E. nana* and *E. latitans*), at the light-microscopy level, labeling PAX6 with a polyclonal antibody and F-actin with phalloidin stain. Confocal microscopy, followed by analysis in CellSens software, was used to image and quantify colocalization between the two proteins. In zebrafish, whole-mount labeling with PAX6 polyclonal antibody and DAPI counterstain was used to assess PAX6 localization.

Results: Confocal microscopy, along with CellSens imaging software, revealed partial overlap of PAX6 with F-actin within the actin-rich stereociliary bundles at the hair-cell apical surface of neuromasts (n=7) in Eurycea salamander larvae, with *E. nana* at 2 months post-oviposition and *E. latitans* at 3 months post-oviposition. Interestingly, whole-mount immunohistochemistry of 5-dpf zebrafish larvae localized PAX6 to the neuromast mantle cells.

Conclusions: Our primary results of colocalization analysis in Eurycea salamanders support the hypothesis that PAX6 could have functional significance in the actin cytoskeleton of the stereociliary bundle of hair cells. Currently, we are employing transmission electron microscopy to gain more insight into PAX6 association with the actin cytoskeleton in this region. Moreover, PAX6 labeling in the mantle cells of zebrafish warrants further investigation, as mantle cells have been reported to contribute to hair cell regeneration after damage through a transient state. These observations motivate ongoing studies to determine whether PAX6 activity in mantle cells influences hair cell regeneration and whether PAX6 plays a broader role in sensory biology by potentially associating with actin filaments in the stereociliary bundle.

M88. In Vivo Zebrafish Screening Identifies Potential Ototoxins Among Novel and Repurposed COVID-19 Therapeutics

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Background: Current drug toxicity screening protocols for newly developed or repurposed therapeutics rarely assess ototoxicity, potentially overlooking harmful side effects that can lead to hearing loss. This oversight became especially concerning during the COVID-19 pandemic, when the urgency of drug development and repurposing left many compounds insufficiently evaluated for auditory toxicity. To address this challenge, a model is needed that can rapidly and reliably assess hair cell toxicity. The larval zebrafish lateral line provides a robust model, with its

externally located hair cells that are homologous to mammalian inner ear hair cells, enabling rapid in vivo assessment of drug-induced damage through bath immersion exposure.

Methods: An in vivo larval zebrafish model was used to screen a COVID-19 drug library of more than 300 compounds for ototoxic potential. Hair cell nuclei of five day-old zebrafish were labeled with Yo-Pro-1 dye and exposed to one of three concentrations of candidate drugs via bath immersion for 2 or 24 hours. To strengthen scientific rigor, compounds identified as potential ototoxins in the primary screen underwent secondary validation utilizing transgenic Brn3c:GFP zebrafish, which express GFP in lateral line hair cells. Following a 24 hour drug exposure, hair cell counts were obtained from five neuromasts per fish and compared to DMSO controls using statistical analysis.

Results: Of the 306 compounds assessed in the primary screen, 74 showed potential ototoxic effects. The secondary validation further confirmed 36 compounds as hair cell toxins, including drugs like nicotine, the anti-malarial drug quinine, and the anti-fibrotic agent ezurpimtrostat.

Conclusions: We identified 36 compounds with potential ototoxic effects in our zebrafish screen. These compounds are now being evaluated for general cytotoxicity in zebrafish, with non-cytotoxic candidates advancing to rodent models to assess translational relevance in a mammalian system. Future studies will also incorporate inflammatory conditions to better mimic COVID-19 symptomology. This work underscores the importance of incorporating ototoxicity screening early in drug development and highlights the clinical relevance of zebrafish as a translational model in auditory research.

M89. Characterization of Mechanosensitive Ion Channel and Lipid Scramblase Properties of TMEM63B Pathogenic Mutations

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Background: TMEM63B is a member of the OSCA/TMEM63 family of mechanically activated (MA) ion channels and is structurally related to the Ca²⁺-activated lipid scramblase TMEM16 and the transmembrane channel-like (TMC) proteins. Recent evidence indicates that TMEM63B functions both as a MA channel that mediates non-selective cation currents and as a lipid scramblase capable of externalizing phosphatidylserine (PS) to the outer leaflet of the plasma membrane. Pathogenic point mutations in TMEM63B are associated with a range of neurological disorders in patients, including developmental and epileptic encephalopathies, neurodegeneration, and in some cases, progressive hearing loss. However, the molecular mechanisms by which pathogenic variants disrupt the dual functions of TMEM63B remain poorly understood. Furthermore, homozygous TMEM63B knockout (KO) mice (*Tmem63b*^{-/-}) exhibit outer hair cell (OHC) degeneration and profound deafness. We therefore hypothesize that unexplored molecular features of TMEM63B, beyond its reported role as an osmosensor, may contribute critically to auditory function.

Methods: In this study, EGFP-tagged wild-type and mutant forms of TMEM63B channels were expressed in HEK293T cells. The protein expression and localization patterns of TMEM63B were examined by confocal microscopy. To characterize the ion channel properties of

TMEM63B mutants, mechanosensitive currents were recorded in cell-attached patches with a high-speed pressure-clamp system. Lipid scramblase activity as measured by PS externalization was assessed with Annexin V-based fluorescent assays. Furthermore, we generated several TMEM63B-related mouse models, including KO and knockin (KI) lines -specifically, a reporter line expressing TMEM63B-mScarlet3 (Tmem63bmScarlet3/mScarlet3) -to investigate TMEM63B localization pattern and its functional role in auditory physiology.

Results: We found several pathogenic mutants that retained the ability to form functional mechanosensitive channels, as evidenced by detectable MA currents when expressed in HEK293T cells. One mutant generated MA currents similar to those of wild-type TMEM63B, however, most variants exhibited reduced current amplitudes in cell-attached recordings. Similar to previous reports, our data from Annexin V-based microscopy assays indicated that the majority of TMEM63B mutants displayed increased levels of PS externalization to varying degrees, with the exception of one that did not enhance PS exposure. Using the Tmem63bmScarlet3/mScarlet3 reporter mice, we validated TMEM63B localization in both inner and outer hair cells. Auditory function assessment via ABR and DPOAE demonstrated hearing impairment in homozygous KO Tmem63b/- animals. In contrast, Tmem63b+/- heterozygous and Tmem63bmScarlet3/mScarlet3 reporter mice exhibited normal hearing thresholds.

Conclusions: Our study demonstrates that multiple pathogenic point mutations in TMEM63B preserve mechanosensitivity while resulting in diminished current amplitudes and promoting aberrant PS externalization. These functional alterations may underlie the molecular mechanisms of TMEM63B-related channelopathies. The mouse models generated herein also provide valuable tools for further elucidating the physiological and pathological roles of TMEM63B in hearing and beyond.

M90. Current Methods for Human Temporal Bone Research: From Celloidin Section Staining to Tissue Clearing Techniques for 3D Immunolocalization

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Background: Traditional human temporal bone (HTB) processing employed celloidin embedding and serial sectioning methods to evaluate histopathology for clinico-pathologic correlations. Other embedding methods to study the human inner are paraffin embedding, plastic-resin embedding or OCT embedding to obtain cryostat sections. Auditory and vestibular endorgans are microdissected from the temporal bone and embedded or stained for whole mount preparation. These methods are used for immunohistochemistry (IHC) and immunofluorescence (IF), DNA extraction and RNAScope. In this work we present the workflow to optimize the use of HTB for the identification of multiple proteins and RNA, as well as the 3D structural organization using clearing techniques.

Methods: HTBs obtained at autopsy were used for this study (UCLA IRB protocol # 22-001587). Postmortem time was between 10-24 hrs. Twelve HTBs from donors with normal hearing and balance, ages 60 to 90 years old, 6 males and six females were used. Archival 20-micron thick celloidin sections were used for IHC, double labeling and multiplex IF, histopathology and 3D reconstruction. Whole cochlea and vestibular endorgans were micro

dissected from decalcified temporal bones and used for: a) Paraffin embedding (low melting point paraffin), 5-micron thick sections were obtained and used for histology, IF or in situ hybridization (RNAScope), b) whole mount IF staining c) Micro-CT scan and correlative histopathology, and d) tissue clearing techniques for 3D-IF. For micro-CT and clearing techniques the undecalcified bone that surrounds the endorgans was removed. Light, laser confocal and light sheet microscopes were used for histological and IF analysis.

Results: Celloidin sections were useful for double IF staining and multiplex IF staining (up to 5 immunomarkers) as well as for traditional histopathological analysis. Paraffin sections were useful for IHC and IF, as well as for RNAScope. Whole mount staining allows to visualize hair cells and nerve fibers in the organ of Corti or utricle using IHC or IF. Microdissected endorgans embedded in plastic were useful for light and TEM analysis. Twenty-micron thick CT images were obtained with the Micro CT allowing the visualization of the human cochlea and vestibule to be used for correlative analysis of H and E sections from the same specimen. Clearing methods allow the spatial visualization of neurons and fibers using beta-tubulin and light sheet microscopy.

Conclusions: Traditional histological techniques and modern methods can be applied to investigate the human cochlea and vestibule, importantly a single temporal bone can be used for different types of staining. Multiplex IF staining allowed the visualization of several proteins using the same tissue section. Cleared IF staining whole cochlea and endorgans allow the 3D visualization of the cellular structures using light sheet microscopy.

M91. Localization of Tomoprotein in the Human Cochlea

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Background: Cochlin is a protein abundantly expressed in the inner ear. Cochlin-tomoprotein (CTP, tomoprotein), which is generated by cleavage of its N-terminal LCCL domain, has been reported as a diagnostic marker specifically detected in the perilymph fluid. Although there have been reports about the localization of cochlin in human cochlea, the specific localization of tomoprotein is unknown. The aim of this study was to investigate the distribution of tomoprotein in inner ear tissue by immunofluorescence (IF) staining using human temporal bone celloidin sections.

Methods: Celloidin-embedded sections were obtained from 16 human temporal bones (8 male and 8 female ages 50 to 85 years old, normal hearing and balance) (UCLA IRB# 22-001587). Celloidin was removed from the sections by immerse them in sodium ethoxide, rehydrated and immersed in antigen retrieval acid solution (Vector Labs), incubated in trypsin solution (Abcam Trypsin kit). Sections were incubated in blocking solution containing 0.5 %bovine serum albumin and 0.01% Triton X-100. Following by the incubation of the rat monoclonal antibody against cochlin/COCH and rabbit anti S-100 polyclonal antibody against S-100 protein, secondary fluorescent antibodies against the primary antibodies were used to visualize the immunoreaction. Laser confocal microscopy images were obtained from the basal, middle and apical turn of the cochlea.

Results: Tomoprotein-IF was detected in type I, type III and type IV fibrocytes of the spiral ligament at the basal turn, middle and apical turn with a trend towards less tomoprotein IF-signal in the basal turn than in the middle and apical turns. S-100 protein allows the visualization of the fibrocytes in the spiral ligament. Tomoprotein-IF staining was also observed in spiral limbus. These distribution patterns were like previous reports for cochlin-IF and COCH mRNAs in the human cochlea.

Conclusions: Cochlin tomoprotein-IF was consistently present in fibrocytes of the spiral ligament and cells of the spiral limbus. These findings suggest that tomoprotein may be involved in the maintenance of extracellular matrix of the human cochlea.

M92. Cross-Species Investigation of the Mammalian Olivocochlear Efferent System: Structure-Function Correlates

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Background: The auditory efferent system provides descending control of hearing function via feedback loops that link central processing centers with peripheral hearing structures in the cochlea. Modulation of mammalian cochlear responses is mediated by two populations of brainstem olivocochlear (OC) neurons: the lateral OC neurons that synapse onto the type 1 auditory fibers near inner hair cells and the medial OC neurons that innervate outer hair cells. The OC system is hypothesized to facilitate detection of signals in noise, auditory selective attention, and protection against accrual of damage from noise exposure. Here we compare cochlear efferent innervation patterns with measurements of hearing function obtained in diverse mammals to characterize how variation in OC innervation may underlie variable resistance to noise across species.

Methods: We conducted a systematic review and meta-analysis compiling histological studies of the anatomy of the efferent OC system and functional assessments of auditory susceptibility to noise, including critical ratios, minimum audible angles, and auditory nerve responses to signals in noise. We performed immunolabeling in cochlear and brainstem tissue to quantify efferent innervation patterns in echolocating bats, mice, humans, and non-human primates. Using new and published quantitative data from model and non-model species, we performed a comparative analysis evaluating trends in behavioral and physiological hearing metrics with olivocochlear innervation patterns.

Results: We observed correlations between OC innervation, including cell counts and LOC and MOC synaptic terminal density in the cochlea, and noise susceptibility, with evidence for anatomical specializations that may support enhanced resistance to noise in auditory specialist species (e.g., echolocating bats) compared to generalists (e.g., rodents).

Conclusions: Cross-species variation in the olivocochlear system provides insight into susceptibility to noise and age-related hearing loss due to accrued noise damage. We highlight key findings in OC innervation patterns observed in species that appear to be resistant to both noise-induced and age-related hearing loss. The aggregated data on olivocochlear anatomy and innervation presents a useful reference for continued comparative studies of the mammalian auditory efferent system.

M93. Three-Dimensional Temporal Bone Imaging and Reconstruction in Caspase-3 Transgenic Mice

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Background: Recent advances in lightsheet fluorescence microscopy allow for three-dimensional (3D) volumetric imaging of the inner ear with preserved spatial context. The objective of this study is to compare inner ear anatomy in mice using 2D whole-mount immunostaining and 3D reconstruction of the whole temporal bone with inner ear organs to identify anatomical differences between the different techniques. We used Caspase-3 (CASP3) transgenic mice which exhibit normal organ of Corti development initially with progressive degeneration of auditory hair cells, spiral ganglion neurons, and hyperplasia of supporting cells, leading to severe hearing loss and balance dysfunction.

Methods: Temporal bones from CASP3+/+, CASP3+/-, and CASP3-/- mice (ages 1-3 months) underwent dissection, decalcification, and urea-based tissue-clearing protocol. Whole mount cochlear and vestibular tissue immunostaining was performed to label hair cells (MYO6, phalloidin), endothelial cells (CD31) and neuronal cells (NF-H, β -tubulin). Samples were imaged using fluorescent microscopy for 2D whole mount tissue and lightsheet microscopy for 3D rendering of tissue-cleared whole temporal bones. Analyses focused on cochlear hair cell distribution, vestibular organ morphology, and spatial orientation.

Results: Both 2D and 3D imaging techniques consistently demonstrated clear resolution of three rows of outer hair cells and a single row of inner hair cells. Whole-mount microscopy revealed subtle differences in staining intensity, including increased phalloidin-positive vestibular hair cells in CASP3+/- mice, consistent with prior reports of supporting cell hyperplasia. Intriguingly, although previous literature describes an approximate 90-degree angle between the utricle and saccule, 3D lightsheet imaging uncovered a novel spatial variation in one CASP3+/- mouse in which the utricle and saccule appeared nearly parallel in orientation. This architectural anomaly was not detectable using traditional 2D methods.

Conclusions: Our findings demonstrate the complementary value of 2D and 3D imaging for analysis of intricate spatial and structural relationships in temporal bone anatomy. While 2D microscopy remains standard for high-resolution cellular characterization, 3D lightsheet microscopy provides superior spatial context, enabling discovery of anatomical variations not otherwise appreciable with 2D imaging techniques. Together, these methods revealed both cellular differences and novel vestibular orientation abnormalities in CASP3+/- mice. This data underscores the utility of 3D imaging for investigating the entire temporal bone spatial characteristics in the audiovestibular dysfunction mouse models and serves as the foundation for future quantitative studies.

M94. A Non-Invasive Test for Stria Vascularis Dysfunction

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Background: Age-related hearing loss is common in the human population but it is highly heterogeneous in aetiology which has hampered efforts to develop ways of stopping its progression. Three major sites of the initial dysfunction are the sensory hair cells, their innervation, and the stria vascularis which generates the high potassium endolymph maintained at a high endocochlear potential bathing the apical surface of hair cells. Treatments aimed at the initial site-of-lesion may be useful, and diagnostic tools to distinguish the primary site would help stratify clinical trials and facilitate selection of the most suitable treatment for each person. Our aim is to find a non-invasive diagnostic tool that can be validated using mice in which we know the primary sites-of-lesion and translated to a human test.

Methods: A panel of mouse mutants was used including two with stria vascularis dysfunction and reduced endocochlear potential (S1pr2 < stdf > and Sgms1 < tm1b >), one with sensory defects (Slc26a5 < tm1 > , affecting outer hair cell function) and one with a neural/inner hair cell defect (Klhl18 < lowf >). All were analyzed at an age when ABR thresholds were elevated but there were minimal secondary changes such as hair cell degeneration that would complicate interpretation. Littermate controls were analyzed alongside the mutant mice at the same ages. In addition to standard ABR tests, we used the same electrode configuration to record the individual trial-level responses to 512 stimuli in order to calculate inter-trial phase coherence (ITC), a unitless measure of the phase coherence of the trial-level ABR responses. The maximum ITC in a 2-ms window around the peak of ABR wave 1 was plotted with respect to the corresponding mean ABR wave 1 amplitude and results were analyzed using k-means clustering, an unsupervised machine learning tool.

Results: Mice with reduced stria function showed good ITC compared with the amplitude of the averaged ABR wave 1 response amplitude, similar to the distribution within the k-means clusters of the littermate control groups. In contrast, mice with sensory or neural deficits showed poor ITC. The mice showed a range of ABR thresholds but these did not add to the discrimination provided by ITCmax/ABR wave 1 amplitude values.

Conclusions: This technique represents a new non-invasive test that distinguishes mutant mice with known stria vascularis dysfunction and reduced endocochlear potential from mice with sensory hair cell or neural defects but with normal endocochlear potential. This method might be useful in humans to predict whether they have reduced stria function, which cannot be measured directly, and to identify who would benefit from treatments aimed at boosting stria function.

M95. Cell-Type Specific Dependence on Gjb2 Expression for Gap Junction Coupling in the Developing Cochlea

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Background: A morphologically and genetically diverse population of non-sensory supporting cells surround hair cells within the inner ear and are critical for ion, metabolite, and neurotransmitter homeostasis, structural scaffolding, and generation of pre-hearing spontaneous activity. These supporting cells are extensively coupled to one another via gap junctions, and genetic variants which disrupt gap junction function underly the most common forms of congenital hearing loss. Despite their broad expression, mechanisms by which disruption of gap

junction proteins in non-sensory cells leads to a spectrum of hearing loss phenotypes is incompletely understood.

Methods: We manipulated expression of connexin 26 (Cx26), encoded by the gene *Gjb2*, in mice using a knock-in lines (Tecta-Cre, NPY-Cre) to restrict deletion of Cx26 to cochlear supporting cells (Cx26 cKO)

Results: Loss of *Gjb2* results in failed maturation of supporting cell cytoskeletal architecture despite proper developmental fate specification and cell survival into early maturity in Cx26 cKO mice. Unexpectedly, whole cell patch clamp recordings in excised P7 cochleae across distinct supporting cell subtypes revealed a differential dependence on Cx26 for gap junction coupling. Inner phalangeal cells, inner border cells, and lateral Kölliker's organ demonstrated largely preserved intercellular coupling in Cx26 cKO cochleae, as well as generation and propagation of spontaneous activity and calcium. In contrast, inner and outer pillar and Deiters' cells required Cx26 gene expression, but not that of connexin 30, for intercellular biochemical coupling, propagation of electrical signals, and structural maturation. Hensen's and Claudius cells similarly depended upon Cx26 expression for electrical and biochemical gap junction coupling. Next, we used single cell RNA sequencing databases to identify cell-type specific gene expression patterns to selectively remove Cx26 from individual subtypes hypothesized to be critical for sensory epithelium maturation. We removed Cx26 expression specifically from inner pillar cells (IPCs) within the developing sensory epithelium using an NPY-Cre mouse line (IPC-Cx26 cKO). This targeted manipulation resulted in impaired gap junction coupling between medial and lateral supporting cell populations and biochemically isolated IPCs, confirming their reliance on Cx26; however, this uncoupling unexpectedly failed to disrupt cytoarchitectural maturation of the sensory epithelium, trigger hair cell degeneration, or impair acoustic sensitivity. These results suggest that loss of gap junction coupling within a single subtype of supporting cells was insufficient alone to induce the detrimental effects of sensory-epithelium wide syncytial disruption.

Conclusions: Together, these findings highlight that specific cell types within the cochlea depend on Cx26 for intercellular coupling, but in conjunction are critical for subsequent structural maturation of fluid-filled spaces within the cochlea. These findings have important implications for development of precise therapeutic interventions to restore hearing, as only specific cell populations may require Cx26 expression at discrete developmental timepoints to enable functional recovery, while others may maintain coupling sufficient for developmental processes through compensatory mechanisms.

M96. Endocochlear Potential Contributes to Hair Cell Death in TMPRSS3 Hearing Loss

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Background: TMPRSS3 is commonly implicated as a cause of human hearing loss. Bi-allelic pathogenic variants in TMPRSS3 cause two forms of sensorineural HL: early-onset severe to profound HL (DFNB10) as well postlingual progressive high frequency HL (DFNB8). In the inner ear, TMPRSS3 is expressed in inner hair cells (IHCs), outer hair cells (OHCs), supporting cells, and in type II, but not type I SGNs. The previously developed Tmprss3-null mouse model (Tmprss3Y260X/Y260X) exhibits normal hair cell development, but experience rapid hair cell death from P12 to P14 at the onset of hearing. The mechanism for hair cell death at the onset of hearing in Tmprss3Y260X/Y260X mice is not understood. However, due to the coincident timing of normal development of the endocochlear potential (EP) and rapid hair cell degeneration in Tmprss3Y260X/Y260X mice, we hypothesized that EP may play a role in hair cell loss and subsequent deafness in Tmprss3Y260X/Y260X mice.

Methods: We performed detailed EP measurements of Tmprss3Y260X/Y260X mice from P7-P24. We performed cochlear explants at P7 prior to HC degeneration followed by in vitro cultures for up to 28 days to assess HC survival. To further investigate the role of the EP on TMPRSS3-mediated hair cell death in vivo we crossed the Tmprss3Y260X/Y260X line, with the two different mutant mouse lines, Mitf⁺/Mi-wh and Pou3f4delJ. Next, we performed pharmacologic reduction of EP in vivo using furosemide from P11-P14.

Results: We demonstrate that Tmprss3Y260X/Y260X mice display an early and temporary spike in endocochlear potential (EP) prior to the onset of hair cell death followed by EP levels like controls after P14. We observed near complete hair cell survival with in vitro cochlear explants from Tmprss3Y260X/Y260X mice. In vivo studies also demonstrated complete preservation of all cochlear hair cells regardless of sex in double mutant mice (Tmprss3Y260X/Y260X;Mitf⁺/Mi-wh and Tmprss3Y260X/Y260X;Pou3f4delJ). Furthermore, systemic administration of furosemide, a drug that reduces EP in vivo, reduced hair cell death in Tmprss3Y260X/Y260X mice.

Conclusions: These findings suggest that extracellular factors, including EP, play a role in TMPRSS3-related hair cell survival and hearing loss, and suggest that modulating EP could be a therapeutic strategy.

M97. Probing the Role of Lateral Olivocochlear Efferent Innervation in Afferent Sound Encoding

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Background: Lateral olivocochlear neurons (LOCs) of the superior olivary complex form efferent synapses on peripheral neurites of spiral ganglion neurons (SGNs) beneath the inner hair cells (IHCs). Unlike the medial olivocochlear (MOC) efferents whose role in outer hair cell (OHC) modulation and noise damage protection is well established, the function of LOCs

remains poorly understood. LOCs release a variety of neurotransmitters to SGN neurites, such as acetylcholine, GABA, dopamine, and neuropeptides like urocortin and calcitonin gene-releasing peptide (CGRP) (Hong et al., 2022). This might allow them to modulate SGN firing, maintain SGN diversity, and provide neuroprotection. However, LOC axons are small and unmyelinated, which makes them difficult to target through lesioning or electrical stimulation, without disrupting MOC innervation to the OHCs (Fuchs and Lauer, 2019).

Methods: Here, we used a genetic approach to functionally disrupt LOC signaling to the cochlea. We developed a SNAP25^{fl/fl}/UrocortinCre (S25UCNcKO) transgenic mouse line to selectively abolish vesicular release from Urocortin-expressing LOCs. We examined cochlear efferent innervation and ribbon synapse morphology with immunohistochemistry. We also analyzed the number and size of synaptic ribbons, as well as the number of afferent synapses in organs of Corti. We measured auditory brainstem response (ABRs) and distortion product otoacoustic emissions (DPOAEs) to assess hearing and OHC integrity. Using in vivo single auditory nerve fiber (ANF) recordings, we assessed spontaneous and sound-evoked responses of single SGNs lacking efferent LOC input.

Results: In S25UCNcKO mice, we observed reduced SNAP25 labeling beneath the hair cells. We did not find significant changes in DPOAE thresholds or amplitudes between mutant and control mice. S25UCNcKO mice had mildly elevated ABR thresholds and lower ABR wave I amplitudes near threshold. However, at higher sound pressure levels, wave I amplitudes recovered, which points to changes in the types of ANFs recruited or disruptions of temporal synchrony near threshold. Ongoing single ANF recordings are testing these hypotheses.

Conclusions: We report a new genetic model aimed to disrupt the function of efferent LOC innervation to the cochlea, by abolishing vesicular release from Urocortin expressing LOCs. S25UCNcKO mice had slightly elevated hearing thresholds and decreased wave I amplitudes, while cochlear amplification by OHCs was intact. These results suggest that LOC efferents might modulate SGN afferent input, excitability and/or synchrony of firing. Ongoing experiments aim to determine whether LOC disruptions lead to changes in spontaneous and sound evoked activity of single SGNs. This work provides a novel tool to dissect the role of LOCs in auditory processing and their contribution to hearing sensitivity.

M98. Bilateral ²³Na MRI of the Rat Inner Ear at 9.4 T Using a Custom Saddle Coil: Feasibility and Optimization

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Background: Ionic homeostasis involving Na⁺ and K⁺ is essential for cochlear transduction; these ions are implicated in various auditory pathologies like aging, noise, and ototoxicity. Still, the direct imaging of these ions in vivo remains technically challenging. Sodium MRI offers a feasible, noninvasive window into epithelial transport and fluid compartment integrity, but sensitivity and spatial resolution are constrained by the short T2 of ²³Na and by coil penetration at the depth of the inner ear and otic capsule. We set out to establish sodium imaging of the rat

inner ear at ultrahigh field, to define acquisition parameters that yield reliable inner ear signaling, and to address hardware limitations that preclude bilateral, higher-resolution inner ear imaging.

Methods: Experiments were performed on a Bruker BioSpec 9.4-Tesla system. Anatomical localization used a proton volume coil, followed by ^{23}Na ultrashort-echo-time (UTE) imaging with a 3 cm surface coil placed over one ear. Pilot parameters were TR/TE = 10/0.31 ms, nominal resolution $0.5 \times 0.5 \times 1.0 \text{ mm}^3$, and a 36-minute acquisition. Observed limitations in cochlear delineation motivated the design and construction of a custom 4.5 cm ^{23}Na saddle coil engineered to improve transmit/receive efficiency at inner ear depth, and to provide bilateral coverage. The study workflow, therefore, comprised initial one-sided pilot imaging with the surface coil, followed by iterative testing of the new saddle coil to enable simultaneous imaging of both ears at equal or finer spatial resolution.

Results: Pilot ^{23}Na UTE scans with the 3 cm surface coil produced inner ear sodium images within 36 minutes, confirming the feasibility of in vivo sodium detection for the inner ear. Image quality in the cochlea proper, however, was limited by depth-related SNR falloff and coverage confined to the ipsilateral ear, constraining consistent visualization of fine cochlear structures. To overcome these limitations, we completed construction of a 4.5 cm ^{23}Na saddle coil tailored for bilateral sensitivity and improved B1 penetration. Preliminary testing with the new coil is underway, with the explicit goal of increasing inner ear image SNR, improving across-ear homogeneity, and enabling higher in-plane resolution while maintaining practical scan times.

Conclusions: Sodium MRI of the rat cochlea at 9.4 T is feasible using UTE acquisitions, but single-sided surface coils impose depth and coverage constraints that limit cochlear detail. A custom 4.5 cm ^{23}Na saddle coil has been developed to address these barriers, with the anticipated benefits of bilateral imaging, improved penetration, and finer spatial resolution. Establishing robust ^{23}Na inner ear imaging provides a foundation for longitudinal studies of ionic homeostasis in models of inner ear pathology and opens a pathway to region-specific, noninvasive biomarkers that can inform mechanisms and interventions relevant to clinical Otolaryngology diagnosis of different types of hearing loss.

M99. Regional Hotspots of Mitochondrial DNA Mutations in the Aging and Stressed Cochlea

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Background: Mitochondrial dysfunction is increasingly implicated in cochlear vulnerability to aging, loud noise exposure and oxidative stress, yet the regional architecture of mtDNA mutations in the cochlea remains underdefined. We aimed to delineate whether mtDNA variants cluster within specific genomic intervals and functional gene loci and to determine how aging and experimentally induced oxidative stress reshape this landscape in vivo and in vitro.

Methods: We performed high-depth whole-mitochondrial sequencing on CBA/CaJ tissue from young adult (n=3), aged animals (n=3), and on stria vascularis derived SV cells treated with (n=3) or without (n=3) hydrogen peroxide. After read trimming and quality control, sequences were aligned to the C57BL/6J mtDNA reference (NC_005089.1) with BWA-MEM; variants

were called using GATK Mutect2 in mitochondrial mode and retained only if depth ≥ 10 . Variants were annotated with predicted functional effects and analyzed at two scales: genome-wide density using 100-bp tiling bins and gene-level burden profiles. Hierarchical clustering of per-gene variant counts was used to visualize cohort separation, and allele-frequency changes were compared within treatment groups and across age cohorts.

Results: Mutations were not uniformly distributed but concentrated within two intervals spanning roughly 4,000–8,000 bp and 14,000–16,500 bp. These hotspots encompassed COX1 (Complex IV) and CYTB (Complex III) and were consistently enriched in both young adult/aged cochlea and H₂O₂-treated SV cells relative to untreated cells. Gene-level clustering corroborated these density findings: COX1 and CYTB carried the highest burdens across stressed/aged groups, whereas multiple NADH dehydrogenase subunits, including ND6, and both mitochondrial rRNAs displayed low, relatively stable variant loads overall, with a couple of standouts. Hierarchical clustering of gene-wise counts clearly separated aged and oxidatively stressed samples from controls. At the variant level, oxidative stress increased the frequency of several protein-altering changes, including a CYTB frameshift near m.15171 and an ND5 frameshift near m.13051. Comparisons between young and old cochlea revealed higher frequencies for selected COX1 and CYTB variants in older tissue, but the overall pattern lacked a uniform directional shift, suggesting that inter-individual mitochondrial backgrounds and stochastic drift contribute substantially to between-animal differences, rather than a simple monotonic increase in total heteroplasmy with age.

Conclusions: Cochlear mtDNA exhibits a non-random mutational architecture characterized by focal hotspots in COX1 and CYTB that intensify with oxidative stress and are evident in aging, while ND subunits and rRNAs remain comparatively spared. This regional vulnerability, potentially reflecting metabolic demand and selective tolerance, offers a mechanistic bridge between mitochondrial genomic instability and impaired cochlear bioenergetics. Mapping these hotspots provides candidate biomarkers of mitochondrial health in the inner ear and points to targeted, region-aware strategies to preserve function, motivating longitudinal studies and interventions that track and modulate hotspot burden over time, which may lead to treatments for age-related and noise-induced hearing loss.

M100. A Zebrafish Model of Cockayne Syndrome Demonstrates Impaired Hair Cell FM1-43 Uptake That Can Be Restored by a Superoxide Dismutase Mimetic

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Background: Cockayne Syndrome is an ultra-rare premature aging condition associated with UV sensitivity, neurocognitive decline, retinopathy, metronidazole-induced lethality, and sensorineural hearing loss. Bi-allelic pathogenic variants in ERCC6 are identified in 70% of affected patients. ERCC6 has been well established to play a role in DNA damage repair, specifically in transcription-coupled nucleotide excision repair. However, little is known about the mechanism of defective ERCC6 function in sensorineural hearing loss.

Methods: In order to investigate the underlying mechanism of disease caused by pathogenic variants in ERCC6, we created an *ercc6* loss of function zebrafish (*Danio rerio*) model. We evaluated survival following UV and metronidazole exposure, measured basal oxygen consumption rates, assessed lateral line mechanoelectrical transduction function by FM1-43 uptake after UV exposure, and performed lateral line hair cell counts. We also treated wildtype controls and *ercc6* loss of function homozygotes with a superoxide dismutase mimetic Mn(III)tetrakis(4-benzoic acid)porphyrin Chloride (MnTBAP) and assessed for survival after metronidazole exposure, measured basal oxygen consumption rates, and evaluated lateral line FM1-43 uptake.

Results: We found that *ercc6*^{-/-} larval viability was significantly reduced following UV exposure. Metronidazole treatment was completely lethal for *ercc6*^{-/-} larvae, with minimal impact on wildtype controls. Oxygen consumption rates were significantly elevated in *ercc6*^{-/-} larvae, potentially indicating altered mitochondrial function. Lateral line hair cells labeled with Alexa Fluor 488 Phalloidin indicated no significant difference in hair cell counts per neuromast following UV exposure compared to untreated controls. Finally, FM1-43 vital dye uptake, a measure of mechanoelectrical transduction function, following UV exposure was significantly reduced. We determined that treatment with a superoxide dismutase mimetic, MnTBAP, resulted in significantly reduced metronidazole lethality, restoration of oxygen consumption rates, and preservation of mechanoelectrical transduction function as measured by FM1-43 uptake.

Conclusions: We propose that defective mitochondrial function and increased reactive oxygen species levels provide a mechanism for hair cell dysfunction in this model of Cockayne Syndrome. These results pave the way for additional exploration of treatment modalities and disease mechanisms for this premature aging condition, using this zebrafish model of Cockayne Syndrome.

M101. Distinct Function of WFS1 in the Inner Ear and Retina: An Insight Into Wolfram Syndrome Pathogenesis

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Background: Variants in WFS1, a gene coding for a protein called Wolframin, have been associated with both sensorineural hearing loss (HL) and optic atrophy (OA). Among the clinically heterogeneous wolframinopathies, Wolfram syndrome (WS) is a severe and rare neurodegenerative disease characterized by juvenile-onset diabetes, OA and HL, as well as a wide diversity of endocrine and neurological alterations. None of the animal models of the WS pathology are presenting with an early onset HL nor OA, impeding the understanding of the role of Wolframin in the visual and auditory pathways. Therefore, we generated a knock-in mouse line, Wfs1E864K, reproducing a human mutation associated with HL and OA.

Methods: Using ABR, DPOAEs and electroretinogram recordings, we analyzed the auditory and visual function of our mutant line. Scanning electron microscopy, transmission electron microscopy and immunohistochemistry assays with various antibodies were then performed to

assess the correlation between electrophysiological dysfunctions and morphological alterations of the neurosensory epithelium of the organ of Corti and retinal specific cell types.

Results: The homozygous mice develop a progressive hearing loss as early as P23 and are profoundly deaf by P29, at all tested frequencies. Likewise, the homozygous Wfs1E864K mice, compared to their WT littermates, have a significant decrease of the positive scotopic threshold response (measuring the function of retinal ganglion cells, RGC), with no alteration of the scotopic wave A amplitude (rods photoreceptors), highlighting RGC abnormalities with no photoreceptor alteration by P30.

The HL is correlated with neurosensory hair cell loss. Interestingly, deafness is concomitant with a severe vestibular syndrome. In addition, we showed that the endocochlear potential of the mutant mice is greatly decreased compared to their wild-type littermates, along with progressive vacuolization and devastating alteration of the stria vascularis. In parallel, we showed that the electrophysiological alterations of the retina function are correlated to a thinning of the inner retina cell layers and a significantly decrease of the RGCs in the mutant mice. Overall, anatomical evaluation of our Wfs1E864K mouse model suggests that visual deficits arise from the RGC alteration.

Additionally, Wfs1E864K mutant protein prevented the localization to the cell surface of the ATP1B1 (ATPase Na⁺/K⁺ transporting subunit beta1), a key protein for the maintenance of the endocochlear potential suggesting that the HL might stem from an unbalanced ionic transfer. Interestingly, in the RGC, we observed a decrease of the number and length of the mitochondria-associated endoplasmic reticulum membranes, an alteration we already put in light in patient's fibroblasts and Wfs1E864K mouse neuronal cell culture.

Conclusions: In summary, we described a new WFS1 mouse model presenting with a profound post-natal HL, associated with a strong vestibular syndrome and severe visual impairment. Taken together, our data suggest tissue-specific roles for WFS1, with sensory alterations stemming from different underlying mechanisms.

M102. Genetic Predisposition to Age-Related Hearing Loss Explains Variation in Middle Ear Muscle Reflex Among Healthy Young Adults

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Background: Although certain monogenic conditions have been shown to impact the function of the middle ear muscle reflex (MEMR), little is known about the genetic basis of its regular functioning. MEMR is regularly measured as part of audiological assessment and is potentially associated with a number of pathologies, including hyperacusis, auditory neuropathy, and cochlear synaptopathy. Better understanding of the underlying biology and associated genetics of the reflex is essential for both basic science purposes and potential clinical applications.

Methods: Puretone- and wideband-elicited MEMR thresholds were assessed in a group of 357 healthy young adults with self-reported normal hearing. 7218 single-nucleotide polymorphisms (SNPs) associated ($p < 5 \times 10^{-6}$) with age-related hearing loss (ARHL) in a previously performed genome-wide association study meta-analysis (meta-GWAS) were selected for analysis (Trpchevska et al., 2023). Linear mixed effects models (LMM) were used to assess the effect of

individuals SNPs on MEMR thresholds. Direction of effect on MEMR thresholds was compared to direction of effect in meta-GWAS using a chi-squared test. SNPs were mapped to their associated genes using ANNOVAR, then principal component analysis (PCA) was performed to create a set of gene PCs (GPCs) for each gene. Effects of GPCs on MEMR were assessed using LMM.

Results: 254 SNPs were significantly associated ($p < 0.05$) with differences in MEMR among young adults with normal hearing. When compared with the results of meta-GWAS, SNPs significantly associated with higher MEMR thresholds were significantly ($p < 0.001$) more likely to be associated with lower likelihood of ARHL in meta-GWAS, and SNPs associated with lower MEMR thresholds were significantly more likely to be associated with greater likelihood of ARHL in meta-GWAS. Of 419 mapped genes, 24 were significantly associated with differences in MEMR thresholds among healthy young adults.

Conclusions: These results show that individual genetic variation associated with ARHL, as well as their mapped genes, are significantly associated with differences in MEMR thresholds among healthy young adults with normal hearing. This suggests that genetic effects influencing the auditory system later in life may also be influencing auditory function from a young age. These results suggest a consistent relationship between SNPs effects on ARHL likelihood and MEMR thresholds. The exact nature of this relationship, as well as the expression of associated genes, are promising directions for future research into the MEMR.

M103. Comparison of Xenium In Situ and Visium HD Spatial Transcriptomics Platforms When Applied to the Mouse Cochlea

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Background: Spatial transcriptomics is a powerful tool that offers untapped insight into tonotopic differences, improved cell-type annotations, and understanding of cell-to-cell communication. Although the inner ear field has adopted transcriptomic techniques such as single-cell RNA sequencing, these methods often struggle in discerning the apical-to-basal origin of major cochlear cell types and medial to lateral discrimination. We previously shared analysis following the application of two new spatial transcriptomic platforms, the Xenium In Situ and Visium HD by 10x Genomics. The Xenium In Situ fluorescently tags genes of interest utilizing common in situ techniques. This platform can analyze up to 5000 genes simultaneously. The Visium HD, however, uses barcoded nucleotides that capture gene expression and are spatially mapped to an image of the tissue. Here, we revisit our analyses comparing the Visium HD and Xenium In Situ with increased optimization.

Methods: We analyzed spatial transcriptomic data from prior Xenium In Situ experiments and optimized our custom gene panel, raising the number of probes specific to lowly expressed transcripts and reducing the number of probes for genes that may be contributing to optical noise. Whole inner ears from C57Bl/6, CD-1, and CBA/J mouse lines were harvested at P8 and P30 timepoints. The tissue was fixed in 4% paraformaldehyde and embedded in paraffin. Cochlear sections of 5-micron thickness were then mounted on either Xenium In Situ or Fisherbrand

Superfrost Plus microscope slides. Tissue for Visium HD experiments was stained with hematoxylin and eosin for imaging. Standard Xenium In Situ and Visium HD protocols were applied to tissue slides. Xenium Explorer, Loupe Browser, and Seurat R package were used for downstream analysis. Transcript density and probe detection accuracy were evaluated for each platform.

Results: Both platforms accurately identified cell-type specific transcript expression. Visium HD was capable of detecting gene expression spanning nearly the entire transcriptome, while the Xenium In Situ platform was limited to a specified gene list. However, the Xenium In Situ's major strength was its sub-cellular transcript detection, as compared to Visium HD's eight square micron resolution which resulted in cell type overlap. A drawback of the Xenium In Situ platform was low transcript detection compared to Visium HD. We attempted to improve transcript detection of the Xenium platform by refining probe set selection and levels by evaluating transcript density from our previous Xenium run and consulting with 10x Genomics bioinformaticians, raising or lowering the amount of probes applied for each specific gene. We also improved cell segmentation by using recently developed cell segmentation kits from 10x Genomics.

Conclusions: We will be presenting our analyses of the Xenium In Situ and Visium HD. We will compare their transcript detection abilities, evaluate the effectiveness of optimization, and discuss the advantages and drawbacks of each platform.

M104. Protein Biophysics-Informed Assessment of Genetic Variants in Hearing Loss

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Background: The Deafness Variation Database (DVD) is a comprehensive, open-access resource from the Molecular Otolaryngology and Renal Research Laboratories (MORL) at the University of Iowa that collects, annotates, and classifies genetic variants associated with hearing loss. It contains over 400,000 missense variants across 224 genes with over 320,000 (~75%) having an unknown significance (VUS). With the emergence of deep learning methods for protein structure prediction, we sought to combine genetic information with biophysical evidence to improve the classification of these variants.

Methods: We applied AlphaFold3 to predict protein structures for all genes and their corresponding isoforms in the DVD. We then optimized these structures with the AMOEBA polarizable force field to resolve steric clashes, repack sidechains and improve backbone conformations. The optimized protein structures were used as input to the folding free energy difference ($\Delta\Delta G_{\text{Fold}}$) prediction tool DDGun; when high, positive $\Delta\Delta G_{\text{Fold}}$ indicates a significant disruption in folding.

Results: Using guidelines set in Pejaver et al. for determining the strength of computational evidence from tools like Revel, we constructed a likelihood function that combines $\Delta\Delta G_{\text{Fold}}$ and Revel, and used Bayesian analysis to identify those VUSs very likely to cause hearing loss. The Bayesian formulation established by combining $\Delta\Delta G_{\text{Fold}}$ with Revel identified ~20,000 missense VUSs with very strong evidence of being deleterious at a false positive rate of < 0.1%.

Conclusions: Using deep learning protein structure prediction models, sequence-based genetic predictors, and biophysical evidence, we identified missense variants highly likely to lead to protein misfolding. Future work will link these data to patients who carry prioritized missense variants but did not receive a definitive genetic diagnosis for their hearing loss to determine whether variant classification can be upgraded from VUS to likely pathogenic.

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M105. Genome-Wide Association Study of Hearing Loss by Frequency and Principal Components in Meta-Analysis of Million Veteran Population and United Kingdom Biobank

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Background: The worldwide burden of health loss due to all forms of hearing impairment is estimated at 43.4 million Years Lived with Disability (YLDs), representing 28% of cumulative YLDs due to all physical impairments, predicted to increase 50% over the next 30 years. Yet, sensorineural hearing loss (SNHL) is permanent, and while assistive devices can address a hearing deficit, only one-third of those dispensed are used, and use is socio-economic- and race-specific. Genome-wide association studies of hearing loss have thus far identified approximately 6-7% of the estimated 36-67% heritability of SNHL. Here we analyze GWAS in the Million Veteran Program using phenotypes of standard audiogram frequencies as well as the first principal components (PCs) of the audiogram to identify potential variants, genes, and pathways related to auditory features.

Methods: Frequency thresholds and four PCs from 278,267 participants with standard frequency audiogram data served as the phenotype. Genotyping, quality control, imputation, and ancestry determination were performed for each frequency threshold and the first four PCs of the audiograms. GWAS was performed for each frequency and each ancestry group separately in PLINK 2 as a linear regression, then meta-analyzed in METAL, for a total of 48 GWAS'. Functional mapping and annotation, gene-based and gene set analysis were implemented in FUMA with MAGMA. SNP-based heritability for each frequency and PC was estimated. Identified genes were compared with hereditary hearing loss genes.

Results: 240,268 participants had complete frequency information. Heritability ranged from 5.4% for 250 Hz to 12.1% at 4000 Hz and constituted 10.1% of PC1. 36 individual GWAS plus 8 meta-analyses elicited 448 unique loci. In the multi-ancestry GWAS, 113 mapped genes contained SNPs with CADD scores > 12.37 of which 4 were exonic, and 5 within UTR regions. MAGMA gene-based analysis identified 849 significant genes. Gene-set analysis in multi-ancestry identified significant gene ontology terms which varied across frequencies and PCs. A broad overview revealed lower frequencies to be significant for stereocilial structure and function, with higher frequencies engaging in sensory organ morphogenesis, elongation, and differentiation, however, there is much overlap in the frequency-significant gene sets. Comparison with 193 genes listed at HereditaryHearingLoss.org indicates that 29 genes are significant in meta-analysis (15.0%).

Conclusions: This is the first large-scale GWAS based on an audiologic phenotype demonstrating the statistical power of the Million Veteran Program audiologic data. We identified 448 unique loci, the most of any GWAS to date. Significant pathways include those engaging stereociliary function as well as development and morphogenesis. Future analysis will determine the efficacy of a polygenic risk score as well as relevant pathways that may be amenable to pharmacologic interventions to ameliorate this disorder.

M106. Genetic Factors Influencing the Age of Onset of Presbycusis

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Background: Age related hearing loss (ARHL) is a very prevalent and debilitating disease of aging. There is substantial variation between individuals in the age of onset of ARHL. We hypothesize that heritable factors influence its age of onset. To investigate this, we performed a genome-wide association study (GWAS) of age at first diagnosis of sensorineural hearing loss, in a large cohort of Veterans receiving primary care in the Veterans Affairs hospital system.

Methods: Participants included Veterans from the Million Veteran Program Release 4 with imputed genotype data and linked electronic health record data. We identified all participants with inpatient or outpatient ICD codes for sensorineural hearing loss (ICD9: 388.01, 389.1, 389.2, 389.89; ICD10: H90.3-8, H91.1, and H91.9). As our variable of interest was specifically ARHL, analyses were restricted to include only participants with first diagnosis date made > 1 year after separation from military service. We performed GWAS of age at first diagnosis date in PLINK 2.0, adjusted for age at separation from service, sex, and 10 principal components. Risk loci were annotated using FUMA. Gene-based and gene-set analyses were conducted using MAGMA. Genetic correlation analyses were evaluated using the CTG-Virtual Laboratory database.

Results: Our analytic cohort included 256,383 participants with sensorineural hearing loss (case-only analysis). The average age at first diagnosis was 67 (SD=10 years). GWAS identified 5 genome-wide significant loci, including highly pleiotropic regions such as the 17q21.31 inversion and human leukocyte antigen region. Overall, common genetic variants accounted for 3.5% (SE=0.3%) of the variation in age of diagnosis. Pathway analysis indicated enrichment of associations of genes expressed in inner ear cells ($p=0.004$) and brain ($p=0.0003$). Genetic correlation analyses indicated correlations with self-reported hearing loss ($rg=0.175$, $se=0.04$, $p=2.4 \times 10^{-6}$), noisy workplace ($rg=0.48$, $se=0.06$, $p=2.2 \times 10^{-15}$), as well as 320 other variables related to sociodemographics, cognition, physical, and mental health (all $p < 5 \times 10^{-5}$).

Conclusions: We have performed (to our knowledge) the first large scale investigation of heritable determinants of age of onset of ARHL. Interestingly, while correlation with self-reported hearing loss was relatively weak, results implicate the influence of multiple domains, including environmental exposures that induce hearing loss, sociodemographics, and cognition. Associations with cognitive genetics implicate cognitive/behavioral elements that are commonly associated with ARHL. Associations with sociodemographic variables may indicate risk exposures or patterns that influence treatment seeking. It remains to expand these analyses to other datasets and partition implicated risk loci across domains to better understand how exactly each influences age of onset.

M107. Natural Course of 33 Cases of SLC26A4-Associated Hearing Loss

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Background: SLC26A4-associated hearing loss includes Pendred syndrome and DFNB4, which represent the second most common cause of hereditary hearing loss in Japanese children after GJB2. These disorders are characterized by enlarged vestibular aqueduct and inner ear malformations, sometimes accompanied by vertigo or thyroid goiter. The typical hearing phenotype is congenital high-frequency sensorineural hearing loss with fluctuation and progression. However, long-term follow-up data on SLC26A4-associated hearing loss remains limited. We therefore, aimed to clarify its natural course.

Methods: We retrospectively reviewed patients with SLC26A4-associated hearing loss at our Hospital between 2001 and 2025. Included criteria were: (1) more than two years of follow-up, and (2) audiometric data obtained prior to cochlear implantation. Audiometric assessments were performed using auditory steady-state response (ASSR) and pure tone audiometry (PTA). Mean hearing levels were calculated as the average air-conduction thresholds at 0.5, 1, 2, and 4kHz. The better or worse ears were determined at the initial assessment. Hearing progression was defined as an increase in threshold ≥ 10 dB, while fluctuating hearing was characterized as repeated increase or decrease ≥ 10 dB.

Results: We evaluated 56 ears in 33 patients (12 males, 21 females). The median age at first visit was 9 years (range: 0–90), and the median follow-up was 8 years (range: 2–24). Hearing deterioration occurred in all cases. High frequencies deteriorated earlier than low frequencies, while fluctuations were more prominent in the low-to-mid frequencies. Notably, 80% of fluctuation events occurred before the early twenties; thereafter, hearing progression continued without fluctuation. Most of patients developed bilateral severe-to-profound hearing loss by their late twenties. We identified two common patterns of SLC26A4-associated hearing loss: rapid hearing deterioration and gradual progression, both ultimately severe-to-profound hearing loss.

Conclusions: Herein, we demonstrate that SLC26A4-associated hearing loss progresses over time, with greater fluctuation during childhood and adolescence. After the early twenties, fluctuation becomes less frequent, but both better and worse ears usually advance to severe hearing loss. These findings highlight the need for long-term follow-up and provide valuable information for patients and their families.

M108. Characterization of WFS1 Variants Among Individuals From a South African Population Extend the DFNA6/14/38 Phenotype

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Background: The WFS1 gene encodes wolframin, a transmembrane glycoprotein with ubiquitous expression. Mutations in this gene cause a spectrum of disorders, from the severest

phenotype Wolfram syndrome 1 to isolated hearing loss DFNA6/14/38 at the other end. Between these phenotypes are Wolfram-like syndrome and congenital cataract. Except for Wolfram syndrome 1 (WS1), a recessively inherited condition, all the phenotypes exhibit autosomal dominant inheritance. Missense WFS1 mutations associated with DFNA6/14/38 have been reported in the United States of America, Japan, China, Europe (Swiss, Danish, Hungarian, Finnish, and German families), Taiwan, the Republic of Korea, Iran, and India. Some of the variants have exposed mutation hotspots. High phenotypic variability has been reported, with low to medium frequency configuration in the majority of cases. Interfamilial variability has also been observed.

Methods: We identified six WFS1 missense variants in six individuals with early onset sensorineural hearing loss from an indigenous South African cohort through whole exome sequencing. This cohort of 94 individuals from the Limpopo Province of South Africa had been screened and found negative for mutations in GJB2, the GJB6-D13S 1830 mutation, and the four common mitochondrial mutations A1555G, A7445G, A3243G and A7511C. We undertook variant characterization through an extended comprehensive clinical assessment (audiological, ophthalmological, metabolic, and neurological), CT and MRI imaging, family studies, segregation analysis, and 3D modelling to confirm pathogenicity, establish causality and define the phenotype.

Results: WFS1 variants, c.2146G > A (p.Ala716Thr) and c.1195T > G (p.Trp399Gly) co-segregated with the deafness phenotype and are considered causative of DFNA6/14/38. The audiometric profiles in all our DFNA6/14/38 patients affected all frequencies, were of moderate to severe degree, and worst in the middle frequencies (100 to 2000 kHz). Intrafamilial variability was mild. Two missense variants in a fourth individual c.325C > T (p.His109Tyr) and c.551T > C (p.Met184Thr) were associated with Wolfram-like syndrome features (visual disturbance, balance disorders and sensorineural hearing loss). Her asymmetrical audiogram was severe on the left and profound on the right, affecting all frequencies. Variants c.1538A > C (p.Tyr513Ser) and Variant c.154C > A (p.Pro52Thr) were non-segregating and are considered non-disease causing.

Conclusions: The South African DFNA6/14/38 patients reported in this study differ from reported cases in the severity and shape of the audiogram. In all our cases hearing loss was severe across all frequencies, in contrast to the low frequency hearing loss reported elsewhere. The high phenotypic variability associated with WFS1 variants presents a challenge to variant calling. From our findings, segregation analysis has proved to be the strongest level of evidence for pathogenicity, and the use of an extended clinical assessment approach has provided the accuracy needed for aetiological and phenotypic diagnosis. As we move into personalized treatments for hearing loss, accurate diagnosis will be key to successful interventions and treatment.

M109. Transcriptomic and Proteomic Profiling of a Humanized Mouse Model of Adult-Onset Dominantly Inherited Hearing Loss, DFNA9

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Background: Deafness Autosomal Dominant 9 (DFNA9) is an autosomal dominant (AD) disorder characterized by post-lingual, progressive severe-to-profound hearing loss and vestibular dysfunction. It is caused by mutations in the COCH gene, which encodes cochlin, an extracellular matrix protein abundantly expressed in the spiral ligament and spiral limbus. More than 25 COCH mutations have been identified worldwide, with the p.P51S variant representing the most prevalent cause of autosomal dominant post-lingual hearing loss in Belgium and the Netherlands, where approximately 1500 carriers have been identified. Regarding the pathophysiology of DFNA9, while several hypotheses have been proposed, the exact molecular mechanisms still remain unclear.

Methods: In collaboration with Radboud UMC Nijmegen, a genomically humanized mouse model carrying the Dutch/Belgian Coch p.P51S mutation was developed in our lab, to enable future testing of sequence-specific gene therapies. To characterize this model, a long-term phenotyping study up to 24 months with auditory and vestibular assessment has been conducted. Furthermore, bulk transcriptomic and proteomic analyses were performed on the spiral ligament of 12-month-old humanized wildtype and homozygous mutant mice. To evaluate whether the mutation alters susceptibility to noise exposure, additional groups of wildtype and mutant mice were exposed to high-intensity noise (115 dB SPL for 2 hours).

Results: In the long-term phenotyping study, auditory brainstem response (ABR) and distortion product otoacoustic emissions (DPOAE) data were collected up to 24 months. Notably, sex-differences were observed. Transcriptomic and proteomic analyses revealed differentially expressed genes (DEGs) and proteins (DEPs), with an increased number following noise exposure. Functional enrichment analysis is ongoing to characterize the affected pathways and will be presented.

Conclusions: These findings confirm that the p.P51S mutation induces significant molecular changes, providing novel insights into the pathophysiology of DFNA9 and advancing our understanding of this progressive hearing loss disorder.

M110. Time-Series Transcriptome Analysis of the Older Adult Mouse Cochlea After Noise-Induced Hearing Loss Reveals an Acute Immune Response and Recovery-Associated Pathways

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Background: Noise-induced hearing loss (NIHL) affects approximately 5% of the global population, significantly impairing health-related quality of life, and is of particular interest in the aging population due to its association with an increased risk of cognitive decline. The pathophysiology of NIHL involves a complex interplay of damage to multiple cellular structures within the cochlea. While the pathophysiology of NIHL has been extensively studied in young mice, limited research exists on the molecular mechanisms underlying NIHL in older adult populations. This study provides the first insights into temporal differences in gene expression following noise exposure in older mice with an average age of 17 months.

Methods: We exposed mice to broadband noise of 115 dB sound pressure level (SPL) for 2h and conducted auditory testing before and after noise exposure to confirm the presence of NIHL. Cochleae were collected at 24h, 72h and 1 week post exposure for RNA sequencing (RNA-seq).

Results: Pathway enrichment analysis using the Ingenuity Pathway Analysis (IPA) tool revealed distinct temporal patterns in the cochlear transcriptome. Acute inflammatory pathways were prominently activated at 24h following noise exposure, while pathways associated with collagen degradation, hormone signaling, potassium channel activity and GABA receptor activation peaked at 72h or 1 week after noise exposure.

Conclusions: These findings shed light on the age-specific molecular responses to acoustic trauma, largely consistent with previous experiments in younger mice, and highlight critical windows for cochlear damage and repair. Understanding these temporal patterns in older adults provides information for the development of targeted treatment strategies to mitigate NIHL and its associated risks.

M111. Rodent Cochlear Explant Model: A Robust Ex Vivo Tool for Hearing Research and Otoprotection Studies

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Background: Explant cultures offer a valuable and reliable initial approach for studying drug-induced ototoxicity and potential otoprotective effects. In this work, we developed and validated a refined ex vivo cochlear explant model of ototoxicity induced by cisplatin and aminoglycosides that provides a reliable screening platform for otoprotective compounds. In addition, this model addresses the need for more efficient and ethical approaches to evaluate hearing-damaging effects of therapeutic agents.

Methods: We optimized a cochlear explant culture system using neonatal rodent pups (P1-P4), systematically evaluating critical parameters including rodent species selection (mouse vs. rat), pup age, dissection techniques, and culture medium composition. Explants were exposed to different concentrations of clinically relevant ototoxic agents (cisplatin, kanamycin, gentamicin, tobramycin) for periods ranging from 6 to 72 hours within a standardized 96-hour total culture period. Multiple cellular markers (Myosin 7A, Phalloidin, Hoechst, β -tubulin, CtBP2, V-Glut3, Sox2) were validated using advanced laser scanning confocal microscopy techniques. Robust quantitative and qualitative methods were developed to assess hair cell integrity and loss, nerve fiber and neuron densities across different cochlear explant regions. These methods allowed for comprehensive evaluation of cochlear damage patterns.

Results: For the cisplatin explant model, different patterns of hair cell loss were observed depending on both the dose used and the time of exposure. Cisplatin at 5 μ M with 16 hours of exposure was determined to be the optimal condition to obtain significant hair cell loss while limiting the structural disorganization of the hair cells. Fiber and spiral ganglion neuron density evaluation showed consistent results with the observed hair cell loss patterns. For aminoglycosides, clear dose-response relationships were established for hair cell loss and cellular disorganization. Optimal experimental conditions were systematically determined for each

aminoglycoside tested: 24 hours of exposure for kanamycin at 1 mM concentration, 48 hours of exposure for gentamicin at 60 μ M, and 72 hours of exposure for tobramycin at 50 μ M. These standardized protocols ensure reproducible and consistent experimental outcomes.

Conclusions: Key advantages of our optimized cochlear explant model include rapid screening capabilities, significantly reduced animal usage, and robust quantitative methods to assess ototoxic effects of test compounds. The standardized methodology enables reproducible results across different studies. This approach could accelerate the identification of promising otoprotective compounds before costly but essential in vivo validation and rapidly identify possible ototoxic effects of new compounds under development. Our cochlear explant model provides a powerful, flexible method for studying ototoxicity mechanisms and identifying promising otoprotective agents, ultimately reducing reliance on in vivo testing and advancing translational research for hearing preservation strategies.

M112. Clinical Data-Driven Drug Repurposing for Noise-Induced Hearing Loss Prevention

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Background: The American Public Health Association (APHA) designated noise as a public health hazard. (Banks and Fink, 2022) Noise exposure is the leading cause of acquired hearing loss, a significant global health burden affecting nearly 500 million people worldwide. (WHO, 2018) Overexposure to hazardous levels of noise is estimated to account for 30% of all hearing loss, making noise exposure the leading preventable cause of hearing loss. (Rabinowitz, 2000) Noise-induced hearing loss (NIHL) is also the most prevalent occupational disease in the world. The National Institute for Occupational Safety and Health (NIOSH) estimates that 22 million American workers are exposed to hazardous noise levels every year at work. There are no FDA-approved pharmacological interventions for hearing protection. Noise-induced hearing loss remains a significant, life-altering medical challenge.

Methods: Our objective is to address the unmet medical need through state-of-the-art reverse-translational research approach of drug repurposing. Leveraging publicly available and proprietary large clinical datasets, we have identified a new class of FDA-approved medications for NIHL prevention. The clinical outcomes were further validated using virtual clinical trials based on the retrospective longitudinal patient records.

Results: Computational analysis of the cross-sectional CDC's NHANES dataset identified an FDA-approved drug DXU775529 for the novel indication of NIHL prevention. The discovery was first validated using a zebrafish NIHL model and further confirmed by the computational analysis of the IQVIA's longitudinal medical claims data containing 80 million patients. Not only the results demonstrated the protective efficacy of DXU775529, they also presented the effects of DXU775529 treatment time window, duration, cumulative dose. Other drugs of the same class as DXU775529 were also investigated. Some also exhibited effectiveness for NIHL prevention.

Conclusions: DXU775529 and some drugs from the same class can be very effective for NIHL prevention, particularly when they are taken within days before, on, and after the noise exposure.

Pre-treatment is the most beneficial, but immediate post-exposure treatment can also exert great protective effects. The level of protection is generally proportional to the cumulative dose and duration, but the dosing time windows is the most important factor. This study demonstrates the potential of clinical data-driven phenotypic drug screening and repurposing. DXU775529 and other drugs identified will be further validated in rodent models. It is expected that the novel computational approach will accelerate the drug discovery process towards an FDA-approved pharmacological intervention for NIHL prevention.

M113. Silencing of Ampka Protects Cochlea Hair Cell Against Acute Ototoxicity Damage

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Background: Aminoglycoside antibiotics such as kanamycin (KM) cause irreversible sensorineural hearing loss by damaging cochlear sensory hair cells, particularly outer hair cells (OHCs). Despite extensive research into the mechanisms of aminoglycoside-induced ototoxicity, no clinically effective pharmacological intervention currently exists. Our previous work demonstrated that oxidative stress and AMP-activated protein kinase alpha (AMPK α) activation contribute to noise-induced hearing loss, and that silencing AMPK α (siAMPK α) significantly mitigates hair cell loss and auditory decline. Given the pathological parallels between noise- and aminoglycoside-induced cochlear damage, we hypothesize that targeting AMPK α may offer therapeutic prevention of aminoglycoside-induced hearing loss.

Methods: Using a KM-furosemide (KM-FU) acute ototoxicity model in CBA/J mice, we examined the role of AMPK α in cochlear injury. AMPK α was silenced via siRNA delivered through the round window membrane (RWM) or posterior semicircular canal (PSC). Auditory function was assessed using auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE). Hair cell loss was quantified from myosin-7a immunolabeled surface preparations. Additional assays evaluated kanamycin uptake, mechanoelectrical transducer (MET) channel activity, and stria vascularis permeability.

Results: KM-FU treatment significantly increased AMPK α phosphorylation in OHCs. Delivery of siAMPK α via the PSC preserved hearing thresholds, DPOAE responses and prevented OHC loss. Silencing AMPK α did not affect kanamycin uptake or cochlear permeability, suggesting that the preventive effects were mediated through modulation of intracellular signaling pathways.

Conclusions: AMPK α activation contributes to KM-FU-induced cochlear damage. Pretreatment with AMPK α siRNA via PSC delivery effectively prevents ototoxic injury, identifying AMPK α as a promising therapeutic target for the prevention of aminoglycoside-induced hearing loss.

M114. HBOT-Preconditioned MSC Exosomes Protect Against Cisplatin-Induced Ototoxicity via NOX3 Suppression

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Background: Cisplatin-induced ototoxicity causes irreversible loss of cochlear hair cells and permanent hearing impairment. Although antioxidants and corticosteroids have been explored as potential therapeutic agents, their efficacy and mechanisms remain inconclusive. Hyperbaric oxygen therapy (HBOT) has been reported to enhance tissue repair, attenuate inflammation, promote angiogenesis, and bolster antioxidant effect. Because HBOT can modify cellular physiology and secretory profiles, it may also potentiate the therapeutic properties of mesenchymal stem cell (MSC)-derived exosomes.

This study investigated the therapeutic effects of exosomes derived from HBOT-preconditioned MSCs on cisplatin-induced cochlear injury and explored their underlying mechanisms.

Methods: Exosomes were isolated from MSCs preconditioned with HBOT and applied to mouse cochlear explants prior to cisplatin exposure. Hair cell survival, reactive oxygen species (ROS) levels, and NOX3 expression were quantified. Exosomal microRNA composition was profiled by small RNA sequencing.

Results: Pretreatment with exosomes from HBOT-preconditioned MSCs significantly improved cochlear hair cell survival and reduced ROS generation compared with controls. Cisplatin-induced NOX3 overexpression (> 3 -fold) was markedly attenuated and returned to near-baseline levels in cochlear explants pretreated with exosomes derived from HBOT-conditioned MSCs. Small RNA sequencing revealed 140-fold increase in miR-182, a microRNA implicated in hair cell protection, along with upregulation of miR-199a, miR-27a, and miR-375-microRNAs associated with cochlear development and auditory processes. These results support a mechanistic link between HBOT-induced modulation of exosomal cargo and hair cell preservation.

Conclusions: HBOT preconditioning reprograms the molecular cargo of MSC-derived exosomes, enriching them with protective microRNAs and enhancing their capacity to mitigate cisplatin-induced oxidative stress by downregulating NOX3. These findings provide mechanistic insights into the otoprotective actions of HBOT and identify HBOT-preconditioned MSC exosomes as a promising candidate for the development of novel therapeutics against hearing loss.

M115. Repurposing Cannabidiol as a Candidate Therapeutic Against Noise-Induced Hearing Loss

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Background: Noise-induced hearing loss (NIHL) cases are estimated to be present in up to 24% of American adults, with higher rates amongst males. Previous work from our lab shows that type 1A spiral ganglion neurons (SGNs) within the cochlea may protect themselves from noise trauma by upregulating the pro-survival ATF family of transcription factors and their target genes in response to permanent threshold shift (PTS)-inducing noise. In contrast, type 1C neurons, which lose their synaptic connections during traumatic noise exposure, lack upregulation of the ATF signaling pathway in response to noise exposure. We have identified cannabidiol (CBD), an ATF agonist, as a candidate therapeutic that may extend neuroprotection to vulnerable SGN subtypes and reduce hearing loss following a PTS-inducing noise exposure.

Methods: 9-week-old B6CBAF1/J mice of both sexes were assigned to one of three groups: CBD-only (sham noise), noise-plus-vehicle (sesame oil), and noise-plus-CBD. Mice underwent baseline auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE) testing 1 week prior to noise exposure. At 10 weeks of age, mice were administered CBD (100 mg/kg) twice daily via oral gavage, starting 1 hour prior to PTS noise exposure (102.5 dB, 8-16 kHz, 2 hrs) and continued to received CBD treatment for 3 days following noise exposure. Repeat ABR and DPOAE testing were performed 24 hours and 2 weeks following the noise exposure to assess acute hearing loss and permanent hearing loss, respectively. Following final auditory testing, mice were euthanized by transcardial perfusion to obtain cochlear tissue for histological analyses.

Results: CBD and vehicle-treated female mice showed no significant difference in ABR threshold shifts 24 hours and 2 weeks after noise exposure. However, the ABR threshold shifts from CBD-treated male mice were significantly reduced when compared to the vehicle-treated group at the 24-hour time point, signifying protection from acoustic trauma. DPOAE testing reveals no significant differences in threshold shifts or amplitudes between CBD and vehicle-treated mice of either sex. Histological analysis of cochlear tissue will be performed and presented for outer hair cell (OHC) counts and paired inner hair cell (IHC) synapses along the tonotopic axis.

Conclusions: These data suggest a potential sex difference in the efficacy of CBD as a therapeutic for noise-induced hearing loss. Our experimental paradigm following traumatic noise exposure showed significant protection from ABR threshold shifts in CBD-treated male mice compared to vehicle-treated mice, particularly at higher frequencies 24 hours after noise exposure. However, DPOAE threshold shifts and amplitudes suggest that CBD does not significantly preserve OHC function in either sex. These findings will deepen our understanding of CBD's potential as an otoprotective drug and shed light on sex differences in the molecular basis of hearing loss.

M116. Graded Noise-Induced Hearing Loss Models in Rats: From Transient to Permanent Deficits

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Background: Noise pollution is widespread in today's workplaces, especially in transportation and manufacturing industrial sectors, leading to consequent hearing defects. Noise-induced hearing loss (NIHL) is the most prevalent occupational disease, affecting more than 10% of workers in developed countries. Hearing deficits often start with transient threshold shifts (TTS), characterized by an immediate change in hearing acuity after a single or repeated harmful noise exposure, that recovers over time to pre-exposure levels. More intense or repeated exposures, however, can result in permanent threshold shifts (PTS). Beyond hearing loss, noise trauma can also trigger sensorineural deficits such as synaptopathy, impairing the ability to understand speech in noisy environments, or causing direct cochlear cell damage. This study aimed to characterize the sensorineural effects of six noise exposure intensities in rats, thereby establishing reliable preclinical models of TTS and PTS with histological correlates.

Methods: Anesthetized rats were subjected to a bilateral noise exposure at a noise band of 8-16 kHz, from 97 dB to 112 dB SPL rms by step of 3 dB, for 2 hours. Auditory brainstem responses (ABRs) thresholds, ABRs wave I and Distortion product of otoacoustic emissions (DPOAEs) amplitudes were assessed weekly between baseline (BL) and T+28DAYS, to evaluate hearing impairment. These functional measures were correlated with histological analyses of ribbon synapses and inner (IHCs) and outer hair cells (OHCs) at T+28DAYS.

Results: Animals exposed at 97, 100 and 103 dB exhibited transient hearing loss, demonstrated by significant and sustained impairments of DPOAEs and ABRs thresholds, before retrieving pre-exposure levels twenty-one days after the noise trauma, proportionally to the noise protocol. Wave I amplitudes and latencies were impaired at various frequencies for 97-, 100- and 103-dB groups at several timepoints. Furthermore, ribbon synapses markers decreased from 16 to 32 kHz, proportionally to the noise intensity in 97-, 100- and 103-dB groups. However, ABR and DPOAE results as well as histology observations suggested that the 106 dB noise exposure induced a small but persistent PTS and an even stronger PTS for the 112 dB, followed by OHC loss.

Conclusions: In conclusion, increasing noise intensities in rats induced a graded spectrum of NIHL models, ranging from reversible TTS with isolated synaptopathy to irreversible PTS with cochlear cell loss. These complementary models provide robust preclinical platforms to evaluate otoprotective or regenerative therapies targeting different cochlear pathologies.

M117. From Hair Cell to Neuron Damage: Rat Models of Permanent Noise-Induced Hearing Loss

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Background: Noise is pervasive in today's human lives, from urban noises to loud sounds generated by weapons and explosions. An increasing number of conflicts is being reported worldwide, the largest since World War II, affecting both soldiers and civilians with noise-induced hearing loss (NIHL), while no curative, nor protective treatments are currently available. Severe noise exposure induces cochlear hair cell loss, causing a permanent threshold shift (PTS), alongside spiral ganglions neuron (SGN) degeneration. The objective of this study was to compare two intensities of severe noise exposure to characterize their sensorineural effects in rats, thus providing reliable preclinical models of PTS to test potential otoprotective or regenerative therapeutics.

Methods: Anesthetized male Wistar rats were subjected to a bilateral noise exposure at a noise band of 8-16 kHz, at 115 dB or 121 dB SPL rms, for 2 hours. Auditory brainstem response (ABR) thresholds, ABR wave I and Distortion product of otoacoustic emission (DPOAE) amplitudes were assessed weekly between baseline (BL) and T+28DAYS, to evaluate hearing impairments. These functional measures were correlated with histological analyses, specifically for inner hair cell (IHC) and outer hair cell (OHC) counts at T+28DAYS.

Results: Both exposed groups exhibited permanent hearing loss, demonstrated by significant and sustained DPOAE amplitudes decrease up to 4 weeks post noise exposure. ABR thresholds were dramatically increased with a continuous 20-40 dB threshold shift for the 115 dB group and a 50 dB threshold shift for the 121 dB group, at all frequencies. Wave I amplitudes were significantly decreased by 50 % at the lower frequencies for the 115 dB group, and no longer detectable for the 121 dB group, while wave I latencies were more specifically increased around 8 kHz for the 115 dB group, and no longer detectable for the 121 dB group. Furthermore, OHC counts were decreased for 115 dB animals and both IHC and OHC counts were decreased for 121 dB animals.

Conclusions: Ultimately, a single noise exposure of 115 dB led to a severe and permanent hearing loss characterized by strong threshold shifts, OHC and neuronal defects, therefore providing a relevant preclinical model to test potential otoprotective drugs. On another hand, a single noise exposure of 121 dB induced an even more drastic hearing loss as demonstrated by stronger threshold shifts, complete hair cells and neuronal damages, producing a useful preclinical model to test potential regenerative drugs.

M118. From Transient Hearing Loss to Cochlear Synaptopathy: Preclinical Mouse Noise Models

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Background: Hearing loss is the most common sensory disorder and affects 18% of the population worldwide, but hidden hearing loss remains largely underdiagnosed. Indeed, classical audiometric tests are performed in quiet environments; however, patients often report difficulties understanding speech-in-noise. One of the primary causes of hidden hearing loss is noise exposure, triggering early transient threshold shifts (TTS) with consequent hair cells recovery followed by partial and permanent loss of ribbon synapses between inner hair cells (IHCs), and spiral ganglion neurons (SGNs), often referred to cochlear synaptopathy. The objective of this study was to compare three intensities of noise exposure to characterize their sensorineural effects in mice, thus providing reliable preclinical models of TTS followed by synaptopathy.

Methods: Anesthetized male CBA mice were subjected to a bilateral noise exposure at a noise band of 8-16 kHz, at 97-, 100- or 103-dB SPL rms, for 2 hours. Auditory brainstem response (ABR) thresholds, ABR wave I and Distortion product of otoacoustic emission (DPOAE) amplitudes were assessed before noise exposure, 24h and weekly after noise exposure, up to T+35DAYS, to evaluate the development and progression of hearing impairment. These functional measures were correlated with histological analyses, specifically for ribbon synapse counts with Homer and CtBP2 spots at T+35DAYS.

Results: Both 97- and 100-dB noise exposures induced transient hearing loss, demonstrated by significant but transient impairments in DPOAE amplitudes and ABR thresholds, before retrieving pre-exposure levels within fourteen days after the noise trauma, proportionally to the noise intensity used in the protocol. The 103 dB noise exposure induced a small but persistent permanent threshold shift (PTS) up to T+35DAYS. Wave I amplitudes of the exposed animals were significantly decreased at the highest frequencies proportionally to the noise intensity and

depending on time after noise exposure. By contrast, despite some wave I latency increases early after noise exposure they remained unaffected except at the highest intensities. Furthermore, ribbon synapse counts decreased from 25 to 40 kHz, proportionally to the noise intensity in both 97- and 100- dB groups. The 103 dB noise model presented greater synapse loss.

Conclusions: Ultimately, both noise exposures of 97 and 100 dB induced a transient hearing loss in tested mice, proportionally to the noise intensity of the protocol. These two types of noise-induced TTS provide strong preclinical models to test potential otoprotective or regenerative drugs targeting ribbon synapses between IHCs and SGNs to retrieve normal hearing.

M119. Subclinical Effects of Recreational Noise Exposure: A Pre-Registered Longitudinal Study

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Background: Findings on the subclinical effects of noise exposure have often been inconsistent and inconclusive, likely due to (a) small samples, (b) inaccurate retrospective estimates of noise exposure, (c) post-hoc data dredging, and (d) cross-sectional designs with large effects of between-subject variability. The present study uses: a prospective approach with regular follow-ups (to improve estimates of noise exposure); repeated measures (to minimise unwanted between-subject variability); and pre-registration (to guard against data dredging).

Methods: A cohort of 220 normally hearing teenagers (aged 16-17 at first timepoint) underwent a 3-hour battery of physiological, perceptual, and self-report measures at two timepoints, 3 years apart. Noise exposure was self-reported at 18-month intervals. The primary hypotheses address relations between noise exposure and longitudinal changes in: extended high-frequency audiometry; distortion-product otoacoustic emissions; auditory brainstem response wave I amplitude; and the middle-ear muscle reflex. Exploratory analyses will include: changes in speech perception and tinnitus; effects of sex and skin tone; effects of pattern of noise exposure; effects of nicotine, alcohol, and deprivation; and relations between personal characteristics, physiology, and perception.

Results: Full results will be presented at the conference. At the time of writing, retention rates are over 90% (the study was powered for 60% retention), and it is estimated that longitudinal data will be available from ~200 individuals. The homogeneity of the cohort at baseline (young, otologically normal, with little noise exposure) allows examination of extraneous variability in our measures.

Conclusions: A large-scale longitudinal study on this topic is highly valuable. The data set will be shared openly, and our team welcomes suggestions and collaborations to support exploitation of the data.

M120. Trk Receptor Signaling is Necessary for Normal Inner Hair Cell Synapse Function in Adulthood

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Background: Trk tyrosine kinase receptors and their ligands, the neurotrophins BDNF and Ntf3, are essential for the development and survival of inner ear neurons during embryogenesis. However, their function in adulthood remains unclear. We previously demonstrated that Ntf3 regulates ribbon synapse density in the neonate, its overexpression induces synapse regeneration after acoustic trauma in the young and reduces age-related inner hair cell (IHC) synaptopathy starting at middle age, thus reducing age-related hearing impairment.

To further investigate Trk signaling in the adult inner ear, we examined the consequences of chronic Trk receptor inhibition on auditory function and noise-induced synaptopathy using Larotrectinib/LOXO-101, a Trk inhibitor used for cancer treatment.

Methods: Adult (9-week-old) CBA/CaJ mice were treated with Larotrectinib (100mg/kg/day, gavage) or vehicle (10% DMSO 5% glucose). Noise exposures were at 102dB SPL, 8-16kHz for 2 hours.

Results: Pharmacokinetic studies showed that Larotrectinib reaches the otic capsule, maintaining concentrations above the IC50 for at least 24hrs. To test if Trk inhibition alters normal auditory function, mice were treated daily and ABRs and DPOAEs were measured at baseline and at days 1, 7, 14 and 21 of treatment. Chronic Trk inhibition caused a progressive reduction in ABR P1 amplitudes, initially at high frequencies (32kHz) and later at mid frequencies (> 11kHz) but did not alter hair cell or ribbon synapses numbers (at 22.6kHz), suggesting that the reduced amplitudes reflect reduced synaptic function. Furthermore, the ABR P1 amplitudes recovered after treatment cessation, demonstrating that IHC synapse function requires constant Trk signaling.

To test if Trk inhibition alters the degree of acoustic trauma, two cohorts of mice were exposed to noise and received either Larotrectinib or vehicle treatment for 21 days. For one cohort, drug treatment was initiated immediately after noise exposure. As shown previously, this noise exposure of vehicle-treated animals induced temporary ABR and DPOAE threshold shifts and permanent reductions in ABR P1 amplitudes at mid and high frequencies (hidden hearing loss). In contrast, by day 21 drug-treated mice developed mild permanent ABR threshold shifts and greater ABR P1 amplitude reductions suggesting additive effects.

For the second cohort, noise exposure occurred immediately after the last drug treatment (day 21), and auditory function was evaluated 1-, 7- and 14- and 21-days post-trauma. Surprisingly, in this case, ABR P1 amplitudes in the drug-treated mice recovered better than in vehicle-treated mice, suggesting that Trk inhibition could have attenuated IHC-SGN synaptic function, indirectly protecting them from noise damage.

Conclusions: Trk signaling is required for normal adult auditory function, and its inhibition does not alter IHC or IHC ribbon density but may attenuate their function. The results also suggest that cancer patients receiving Trk inhibitors may be vulnerable to develop hearing problems.

M121. Amitriptyline Treatment of Adult CBA/CaJ Mice: Comparison of ABR Peak 1 Amplitudes After Exposure to Moderate or High Level Noise

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Background: Noise exposure is a major toxic environmental agent to our current lifestyle. Numerous clinical reports and scientific studies have demonstrated that cochlear responses to sound level exposures above 90 dB SPL can range from loss of inner hair cell afferent synapses, to loss of inner hair cell and/or outer hair cell functionality or integrity and can cause moderate to severe hearing loss. No treatment has been so far approved by FDA to treat or prevent hearing loss in the context of noise exposure. We have recently demonstrated that ABR thresholds elevated by high level noise exposure (110 dB x 8-16 kHz x 2 hr) is at least partially protected by intracochlear delivery of Fluvastatin (Depreux et al, 2024). However, the synaptic loss typical of this high level exposure is not protected by the statin. We are theorizing that treatment with a cocktail of two drugs, one that protects synapses and one that protects ABR measured permanent hearing loss would create an improved drug combination for noise exposure.

Methods: As a first step toward this aim, we have compared the effect of Amitriptyline on the ABR Peak 1 amplitudes in adult male CBA/CaJ mice exposed to moderate (100 dB SPL) or high level (110 dB SPL) noise. Amitriptyline, a tricyclic antidepressant, was previously demonstrated to protect synapses from moderate noise exposure (Fernandez et al (2021)). After 1 week acclimation to our vivarium, baseline physiology measures (ABR, DPOAE) were carried out on 11-week-old mice. At 12 weeks, mice were injected IP with a single dose of Amitriptyline (50 mg/Kg), or with carrier, 6 hour prior to exposure to noise. Animals were exposed to octave band noise (8-16 kHz) at either 100 dB SPL or 110 dB SPL for 2 hours. One and two weeks later, ABR was recorded and thresholds and P1 amplitudes were measured. At two weeks, DPOAE thresholds and amplitudes were measured. Cochleas were removed after intracardiac perfusion, decalcified and proceeded to immunostaining for synapses, and hair cells, as described in Depreux et. (2024). .

Results: We were able to confirm a protective effect of Amitriptyline on ABR P1 measures at 100 dB. We found a positive but smaller protective effect of Amitriptyline on P1 amplitudes in animals exposed to high level noise

Conclusions: These data will allow us to progress toward our goal of creating a dual drug combination for the protection of noise induced hearing loss . (Funded by the Office of the Assistant Secretary of Defense Affairs through the Hearing Restoration Research Program under Award number W81XWH-20-1-0484; and the Hugh Knowles Center, Northwestern University)

M122. Establishment of a Neural Inner Ear Trauma Model: A Selective Spiral Ganglion Neuron Injury in a Large Animal Model

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Background: As the primary neurons of the auditory pathway, spiral ganglion neurons (SGNs) are responsible for transmitting auditory signals from the cochlear hair cells to the brainstem's cochlear nuclei, making them indispensable for hearing. Damage to these neurons, whether through mechanical trauma, ototoxic agents or inflammation, can lead to irreversible hearing loss. A detailed understanding of neuronal responses to trauma is therefore essential for the development of novel therapeutic strategies. Ouabain, a specific inhibitor of the Na⁺/K⁺-ATPase, is widely used in small animal models to induce selective SGN degeneration and thereby mimic cochlear neuronal trauma. Hereby, the present study aims to investigate the dose-dependent effects of ouabain on SGNs in a humanoid large animal model.

Methods: Sixteen piglets (n = 4 per dosage group) receive a single intratympanic injection of 0.1, 1, 2.5, or 5 mM ouabain to determine the optimal concentration for inducing selective SGN damage. Hearing function is evaluated preoperatively and repeatedly up to one month postoperatively using auditory brainstem response (ABR) testing. Following functional assessment, inner ears are collected and processed for histology, including mid-modiolar sectioning and hematoxylin and eosin staining, to enable quantitative analysis and comparison of SGN survival across treatment groups.

Results: In our ongoing study, 8 animals (n = 2 per dosage group) have so far been treated with an intratympanic application of ouabain. Preliminary ABR data demonstrate a dose-dependent elevation in hearing thresholds, with higher concentrations resulting in more pronounced and sustained hearing loss across all tested cochlear frequencies. Histological analysis further showed a corresponding reduction in spiral ganglion neuron density on the treated side. In the basal turn, significant SGN loss was detected after treatment with 1 mM (p < 0.0001) and 5 mM (p = 0.023) ouabain compared to contralateral controls, whereas in the middle turn, significant reduction occurred only with 5 mM ouabain (p < 0.0001). Contralateral cochleae showed indifferent SGN densities across all dose groups. The remaining surgeries and histological analyses are planned to be completed prior to presentation.

Conclusions: These preliminary findings support the feasibility of intratympanic ouabain administration as a means to selectively induce dose-dependent SGN degeneration in pigs. Establishing this large animal model offers a robust platform for advancing our understanding of neuronal mechanisms underlying hearing loss and provides a translational basis for the development and evaluation of future therapeutic interventions.

M123. Assessing Auditory Temporal Processing Deficits Affected by Graded Intensities of Noise Induced Cochlear Neural Degeneration

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Background: Noise-induced hearing loss (NIHL) remains a major occupational, military, and recreational health concern. Standard audiometry can detect permanent threshold shifts (PTS) but often fails to detect suprathreshold deficits that impair speech-in-noise (SPIN) perception.

Cochlear neural degeneration (CND)—loss of ribbon synapses between inner hair cells and auditory nerve fibers—is a strong candidate mechanism for this form of “hidden hearing loss”. We aim to understand how PTS and synaptic loss interact after noise exposure to affect the neural encoding of speech-relevant temporal information.

Methods: Here, we assess temporal processing of cues essential for understanding speech, using EEG-like responses obtained to the stimulus amplitude envelope (envelope following responses, or EFRs) and the stimulus temporal fine structure (Frequency modulation following responses or FMFRs) in Mongolian gerbils, a rodent model with human-like low-frequency sensitivity. Young adult gerbils were exposed to a range of noise exposure intensities (100-, 106-, and 118-dB SPL; octave-band noise centered at 1 kHz, 2 hours) to induce progressive damage to the auditory system including synapse loss, PTS or a combination of both.

Results: We found that lower-intensity noise exposures did not result in permanent threshold shifts (PTS), yet 118 dB SPL exposure produced ~20 dB PTS across tested frequencies. Temporal processing deficits, measured using envelope following responses (EFRs), were evident in the PTS group even when stimuli were presented at sensation-level compensated intensities. We observed an increase in EFR thresholds and steeper loudness growth curves, indicating suprathreshold auditory deficits. Additionally, FMFRs, which measure temporal fine structure (TFS) encoding that contributes to speech perception -in-noise (SPIN) were also decreased, suggesting impaired TFS coding at low frequencies following noise exposure. These deficits became increasingly pronounced with higher noise intensities, suggesting an intensity dependent degradation of neural phase-locking to FM cues.

Conclusions: Ongoing work is focused on linking these neural coding impairments with cochlear histopathology. Our findings suggest that EFRs and FFRs provide sensitive, objective indices of peripheral neural injury relevant to SPIN difficulties. These findings deepen our understanding of the peripheral mechanisms underlying temporal processing deficits involved in CND when co-occurring with PTS.

M124. Neurotrophin Therapy for Cochlear Synapse Regeneration After Excitotoxic Trauma

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Background: The cochlear synapses between sensory inner hair cells and auditory neurons in the inner ear are vulnerable to noise-induced excitotoxic damage (cochlear synaptopathy). For patients, this is thought to manifest as difficulty interpreting speech in background noise, despite normal hearing thresholds. Although there is growing concern amongst health experts regarding damaging occupational and recreational noise exposure for current and future generations, there are no pharmaceutical treatment options available to patients who experience milder forms of hearing loss. Brain Derived Neurotrophic Factor (BDNF) and Neurotrophic Factor 3 (NT3) are two neurotrophins that target Trk receptors on the auditory nerves, in turn promoting their development, survival, and repair. These two neurotrophins have shown therapeutic potential in cochlear synapse repair in preclinical models of hearing loss. A key challenge to overcome before clinical implementation of neurotrophin therapy is their complicated and costly manufacturing process. We propose the use of novel, Fc fusion variants of these neurotrophins

(NT3.Fc and BDNF.Fc), which are produced in mammalian cell lines. This project aimed to assess the bioactivity of our novel BDNF.Fc and NT3.Fc neurotrophins to activate their target Trk receptors and repair cochlear synapses after excitotoxic damage using in vitro assays.

Methods: Trk-NFAT-Bla CHO-K1 cell lines that express high-affinity human Trk receptors for either NT3 or BDNF were used to characterise the dose-response relationship and EC50s (dose that produces half-maximal response) of BDNF.Fc and NT3.Fc for Trk receptor activation. These EC50s were benchmarked to those of commercially available native BDNF and NT3 variants, respectively. Postnatal day 2-5 C57BL/6 mouse cochleae were explanted and cultured in kainic acid to simulate excitotoxic damage seen at the glutamatergic cochlear synapse upon excessive noise exposure. The cochleae were treated with NT3.Fc or BDNF.Fc across a range of concentrations. Cochlear synapses were immunostained and imaged, then synapse density quantified for control and treated explants.

Results: Trk receptor assay results demonstrate that BDNF.Fc and NT3.Fc activate their specific Trk receptors and have a lower EC50 (higher potency) than BDNF and NT3, respectively (analyses ongoing). Preliminary cochlear explant results suggest that explants treated with BDNF.Fc or NT3.Fc had higher synapse density relative to damaged, untreated control explants (analyses ongoing).

Conclusions: These results show promise for the therapeutic potential of the novel BDNF.Fc and NT3.Fc neurotrophin variants to activate their target receptors and repair cochlear synapses. If the current trend in the data continues, this project could support further preclinical testing using in vivo animal deafness models and first in human clinical trials. Further research is required to understand whether cochlear synapse repair corresponds to improvements in hearing function following excessive noise exposure.

M125. Functional and Histological Assessment of 3D-Printed Helmet and Hearing Protection Devices in Preventing Blast-Induced Auditory Injury in Chinchillas

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Background: Blast-induced hearing loss remains a significant health concern in military and high-risk occupational environments. Although helmets and hearing protection devices (HPDs) have demonstrated efficacy in mitigating auditory injury from blast overpressure (BOP), the combined protective effect of these interventions has not been comprehensively investigated. The present study investigates the auditory protective efficacy of a custom-engineered, multi-layer 3D-printed helmet, used alone and in conjunction with HPDs, in chinchillas subjected to repeated blast exposures, and aims to investigate functional and histological indicators of auditory injury.

Methods: Thirty-six (N=36) healthy chinchillas (600-800g) were divided into four experimental groups: open (no protection), earplug only, helmet only, and helmet plus earplug. The helmet featured an acrylic outer shell, an acrylate shock-absorbing layer, and a silicone rubber inner lining. Animals were exposed to three consecutive high-level blasts under anesthesia. BOPs were generated using a nitrogen-driven blast apparatus, with pressures ranging 103 and 138 kPa (15–

20 psi). Auditory function was measured pre-blast, immediately post-blast, and on days 4, 7, and 14 post-exposure using auditory brainstem responses (ABR), middle latency responses (MLR), and distortion product otoacoustic emissions (DPOAE). On day 14, animals were euthanized to harvest cochleae for histological analysis. Cochlear whole-mount immunostaining with CtBP2 was performed to assess the integrity of ribbon synapses.

Results: Animals with combined helmet and earplug protection exhibited the lowest ABR threshold shifts and the greatest preservation of Wave I amplitudes across frequencies compared to other groups. Earplug-only animals outperformed helmet-only animals, particularly at mid-to-high frequencies. MLR amplitudes in the dual-protection group remained stable and showed mild recovery, while helmet-only animals displayed greater variability. DPOAE measures indicated partial recovery of outer hair cell function in all protected groups, with the best outcomes in the dual-protection condition. CtBP2 showed significantly higher ribbon synapse counts in the combination of earplug and helmet group.

Conclusions: Dual protection with a 3D-printed helmet and earplugs significantly reduced blast-induced auditory threshold shifts, provides notable protection against blast-induced ribbon synapse loss, preserved both peripheral and central auditory function compared to single-mode or no protection. These findings support the integration of helmet-HPD systems as a promising strategy for mitigating BOP-related auditory injuries.

M126. Real-Time Measurement of Basilar Membrane's Transient Response to Blast Overpressure

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Background: Blast overpressure (BOP) can cause severe auditory injuries, leading to hearing impairments that impact the operational effectiveness of Service members. When BOP transmits into the cochlea, significant motion of basilar membrane (BM) can lead to pathological changes and hair cells damages in the organ of Corti. However, the relationship between BM motion and BOP level has not been quantitatively characterized in experiments under high-intensity blast stimuli. This study measured BM motion in response to BOPs ranging from 26 to 64 kPa (182 – 190dB), using a dual laser Doppler vibrometers (LDVs) system and pressure sensors to simultaneously capture BM motion and BOP transmission in human cadaveric temporal bones (TBs).

Methods: Eight fresh TBs (N=8) were used in this study to investigate BM movement under BOP exposure. The basal turn of BM was accessed through a surgically opened window from the scala tympani (ST). Reflective microbeads were affixed to BM and served as laser targets. The ST was then sealed with a glass slide to maintain the fluid environment and structural integrity. Tympanometry was performed before each blast to ensure that the eardrum was intact. BOP was generated in an anechoic chamber and monitored by two pressure sensors. The sensors were positioned: P0, located laterally to the ear canal entrance to quantify BOP intensity, and P1, surgically inserted into the ear canal before the eardrum. LDV1 measured TB movement as a reference, while LDV2 measured BM movement through the surgical opening. Actual BM

motion was isolated from background motion. All signals were synchronized and recorded at a sampling rate of 1 MHz.

Results: With increasing P0, the peak-to-peak basilar membrane (BM) velocity and displacement rose from 0.2 to 1.3 m/s and from 30 to 310 μm , respectively. The maximum velocities directed toward the scala vestibuli (SV) and ST increased with BOP, ranging from 0.1 to 0.8 m/s. Both BM velocity and displacement exhibited a significant increasing trend with the pressure levels. BM velocity waveforms obtained across different blast overpressure (BOP) levels showed good consistency.

Conclusions: The biomechanical response of cochlea to blast overpressure (BOP) demonstrates a proportional increase in BM motion with rising pressure levels. Consistent directional trends and high repeatability across measurements confirm the robustness of the experimental methodology. These findings indicate that incremental rise in BOP can markedly enhance BM motion, providing important insights into the mechanisms underlying blast-induced auditory injury.

M127. Wide-Range Apical Vibration in the Guinea Pig Cochlea: Bekésy's Observation Suggests the Necessity of the Even–Odd Mode Approach

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Background: G.V. Békésy observed that the apex of the guinea pig cochlea vibrates across a wide frequency range, a phenomenon not fully explained by classical traveling waves on the basilar membrane, whose amplitude decays before reaching the apex ("Experiments in Hearing"). This study reinterprets Békésy's observation within the framework of the even–odd mode theory: odd-mode waves generate traveling waves, whereas even-mode waves propagate to the apex, reflect, and form standing waves that regulate basal excitation.

Methods: A guinea pig cochlea was modeled in COMSOL Multiphysics, solving fluid–structure interaction with the finite element method (FEM). The scala vestibuli (SV) and scala tympani (ST) were combined to form a 3.75-turn spiral tower, 2.6 mm in diameter and 5.1 mm in height. A 16.1-mm basilar membrane with Young's modulus of $E = 1\text{e}9\text{ Pa}$ at the base and $E = 1\text{e}7\text{ Pa}$ at the apex was inserted between SV and ST to reproduce tonotopy up to 50 kHz. The perilymph was modeled as compressible, preserving even-mode propagation. To reproduce the anatomical feature of the guinea pig cochlea protruding into the middle ear cavity, the upper 75% was covered by a conical bone shell ($E = 1\text{e}10\text{ Pa}$).

Results: The model reproduced apical vibrations across all frequencies, showing both axial displacements along the cochlear axis and transverse components perpendicular to it. These patterns were considered to be attributable to structural asymmetry of the cochlear duct. Békésy pointed out in his book that: (1) the movement of the cochlear partition, even when driven with simple sinusoidal motion, extended over a wide area; (2) sometimes these vibrations of the axis of the cochlea led to a longitudinal displacement of the cochlear partition; and (3) this movement was not in phase with the usual vibration of Reissner's membrane. Similar movements to those observed by Békésy were also found in the simulation results.

Conclusions: In a symmetric cochlea, two components are excited: an even-symmetric wave (fast wave) and an odd-symmetric wave (slow wave). When perilymph is treated as compressible and the equations are solved rigorously, the density time-derivative in the continuity equation is preserved, yielding solutions that include both modes. If treated as incompressible, this term is neglected, the even mode disappears, and cochlear mechanics are described only by the odd mode. While this approximation is acceptable at low frequencies, in pathological conditions that break symmetry and enhance mode conversion, or in analyses including high-frequency regions, numerical artifacts may arise, which are not observed clinically. We consider that the apical motion addressed in this study is likewise one of the solutions obtained only when the perilymph is treated as a compressible medium.

M128. Frequency Following Responses Reveal Sensory Loss in the Cochlea

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Background: Sensorineural hearing loss (SNHL) is caused by damage to the cochlea and/or auditory neural components and is the most common type of permanent hearing impairment. While the classical audiogram monitors the “total” SNHL, both distortion product otoacoustic emissions (DPOAEs) and frequency-following responses (FFRs) have the potential to evaluate the health of different elements along the auditory pathway. A major barrier for a more precise clinical application of DPOAEs and FFRs is that their (frequency and level-dependent) correlation with the cochlear mechanics is not fully understood.

Methods: We simultaneously measured DPOAEs, FFRs and, intracochlear responses from the second turn of the gerbil cochlea (best frequency, BF: ~2.5 kHz) using optical coherence tomography (OCT). A total of 25 animals (both male and female) contributed to the study. These three channel recordings, DPOAE+FFR+OCT, were obtained under normal conditions and after modification, either by applying a suppressor or by exposing the cochlea to intense tones that caused damage in different regions. A range of acoustic stimuli (single-, two-, multi-tone) were used to query the effects. Cochlear condition was monitored using DPOAEs.

Results: The FFR were a combination of multiple components that varied in their “modality” (sensory vs neural) and their tonotopic origin. The FFR response to a 2.5 kHz tone at ≥ 50 dB SPL could be suppressed by a wide frequency range of suppressors—spanning more than two octaves—which is consistent with the broad distribution of nonlinearity at the outer hair cell region in gerbil cochlea. The degree of suppression varied with cochlear condition. It remained with a partially damaged cochlea (i.e., the nonlinearity around the traveling wave peak was gone, but the broadly distributed nonlinearity at higher sound pressure levels remained) but diminished in the passive cochlea. FFR responses were also sensitive to cochlear condition and paralleled changes in the intracochlear response. FFR amplitudes sometimes increased post-damage, which could be explained by their distributed cochlear origin, in which damage changed the contribution from local and non-local components differently.

Conclusions: Suppression of FFR responses may serve as a sensitive indicator of cochlear health. For example, a broad suppression pattern corresponds to normal cochlear function, a more localized suppression pattern reflects basal high-frequency damage, while the absence of

suppression indicates widespread cochlear damage. When combined, DPOAE and FFR measurements can extract frequency specific information about SNHL, offering a more precise diagnostic tool for detecting sensory damage.

M129. BAIAP2L2 Modulates Actin Protrusion Shape via Condensate Formation With Espn1 and EPS8

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Background: Stereocilia are actin-rich mechanosensitive organelles in auditory and vestibular hair cells. They require precise regulation of their length and morphology for proper hearing and balance. The mechanoelectrical transduction (MET) complex is localized at the tips of the second row and lower mechanotransducing prolate stereocilia. Stereocilia tip density proteins such as Espn1, EPS8, Myo3A, and the membrane curvature regulator BAIAP2L2, are critical for stereocilia regulation in organ of Corti hair cells. While the localization of BAIAP2L2 at the stereocilia tip is calcium-dependent, Espn1 is not. Both affect MET activity and stereocilia morphology, and a BAIAP2L2 V169A mutation is linked to progressive hearing loss. However, the mechanisms by which Espn1 and BAIAP2L2 precisely regulate stereocilia length and shape, and whether they work in a coordinated manner, remain largely unknown.

Methods: To explore potential mechanisms and regulatory pathways, we performed immunolocalization and co-transfection assays using the COS-7 cell heterologous expression model system.

Results: Our data show that BAIAP2L2 and Espn1 colocalize at the stereocilia tips, with BAIAP2L2 positioned near the membrane and Espn1 located closer to the actin filament bundle. Their distributions are covariant and exhibit substantial overlap, suggesting possible interactions. Using co-transfections assays with constructs encoding selective domains of these proteins, we demonstrate an intricate network of interactions between BAIAP2L2 and multiple components of MET and actin regulatory machineries, which are known to localize at stereocilia tips. Further, co-transfection of BAIAP2L2 with Espn1 or EPS8 in COS-7 cells induces the formation of phase-separated condensates, which originate at filopodia tips and migrate to the cell body. Using immunogold electron microscopy, we localize Espn1 and EPS8 to these condensates. This supports our hypothesis that BAIAP2L2 and Espn1 not only interact directly via SH3 and PR1 domains but also as part of a phase separation and formation of condensates. Additionally, we demonstrate that the BAIAP2L2 V169A mutation, which is associated with hearing loss, reduces the formation of these condensates but does not prevent interactions of BAIAP2L2 with Espn1 and EPS8. Furthermore, we observe that Myo3A transports Espn1 and BAIAP2L2 to the tips of filopodia, resulting in filopodia elongation.

Conclusions: Taken together, these results indicate that BAIAP2L2 colocalizes with Espn1, suggesting an interaction that regulates stereocilia length and tip morphology via specific binding and a phase separation-based mechanism.

M130. Topological Analysis of Time-Domain Cochlear Models Distinguishes Sources of Outer-Hair-Cell Nonlinearity

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Background: The majority of cochlear models operate in the frequency domain, where validation relies primarily on displacement magnitudes and phases. Inherent in such input-output relationships is an assumption of linearity known not to hold in live cochleae; in particular, the classical Fourier transform as an operator exchanging between spectral and temporal data supposes time-domain traces of motion to be well approximated by sinusoids, limiting faithfulness to in vivo outer-hair-cell action on the mechanics of larger-scale structures. Relevant time-domain mechanical analyses have to date largely been confined to those targeting single-hair-cell dynamics (Nadrowski et al. 2004, Faber–Bozovic 2024, among others).

Methods: We develop a multiscale lumped-element time-domain cochlear model, combined in which are a one-dimensional, three-degree-of-freedom representation of organ-of-Corti dynamics (after Simón Gálvez–Elliott 2013) and a three-stage description of outer-hair-cell (OHC) force production (after Kraut–Puria 2024, doi.org/10.5281/zenodo.14576615), the latter incorporating a novel description of the phase relations between hair-bundle mechanoelectric transduction, OHC somatic motility, and cochlear-partition motion. This model is used to simulate time series of structural (basilar-membrane, reticular-lamina, and tectorial-membrane) displacements under stapes forcing, for which purpose a two-dimensional Navier-Stokes-based hydrodynamical model of the cochlear partition (after Beyer 1992) is also implemented. Existing mathematical machinery (from topological mechanics, after Abraham–Marsden 1978, Weinstein 1977) is then borrowed to analyze the composite model’s outputs, for periodic and almost periodic stimuli: we create variants of the model differing in their outer-hair-cell actions (with nonlinearities representing MET gating, prestin saturation, or voltage-dependence of membrane conductance and capacitance) and reconstruct phase-space attractors by Takens-type delay embeddings (after Eftekhari et al. 2018; Botvinick-Greenhouse et al. 2024, arXiv:2409.08767; Kato 2023/25) to obtain qualitative characterizations, alternative to spectra, of each system’s dynamics.

Results: The model variants exhibit distinguishability in phase-space, OHC action itself (if amplificatory) also being detectable by comparison against the baseline passive-model attractor structure. With physiological levels of OHC feedback, reconstructed attractors remain low-dimensional with small local divergence across various schemes for selection of delay time and embedding dimension. Poincaré maps and empirical Floquet multipliers reveal further nonlinearity-specific signatures of possible usage in model design and tuning.

Conclusions: Our results constitute proof of concept for accessing time-domain cochlear nonlinearities by analysis in phase space. The described framework is applicable not only to the simplified model here considered but also to three-dimensional finite-element models developed previously in our lab and to real cochleae (via OCT-vibrometry); in this respect the shortcomings of frequency-domain analysis are avoided, as one may perform model validations strictly with time-series comparanda. Further work in progress considers stochastic extensions of the developed model to assess contributions of biological (Lévy) noise to amplification, compression, and spontaneous emissions.

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M131. 3D Microct-Reconstruction of Human Cochlea for Finite Element Modeling

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Background: Hearing loss due to blast exposure is one of the leading medical issues among service members and veterans. Computational methods, such as finite element (FE) modeling, of the human ear are important tools to investigate and simulate the biomechanical responses of human cochlea during acoustic or blast transmission. However, current FE models of cochlea lack anatomical accuracy at microscale. Micro-computed tomography (μ CT) of cochlea has been done at high resolution, but the successful conversion into a geometrically accurate FE model has not been done due to the lack of mature routine from converting a μ CT reconstructed model into FE model. Here, we established a multi-step FE mesh reconstruction procedure to develop a 3D human cochlea model based on μ CT data, improving the anatomical accuracy of existing computational models and enabling a better understanding of the biomechanical responses to acoustic and blast exposure.

Methods: A cochlea ($\sim 8 \times 8 \times 6$ mm) was dissected from a human cadaver temporal bone specimen (left ear) and fixed in PFA solution. A μ CT scan was applied to the human cochlea at a resolution of $7.8 \mu\text{m}$. To construct the cochlea outer bony walls and osseous spiral lamina (OSL), a repair process, including removal of internal porosity, eliminating noise, and interpolating the center of OSL to make it a continuous panel, was performed. Then, a 2D shell mesh was generated and output as an STL file. Furthermore, the 2D shell mesh was postprocessed with mesh smoothing, mesh decimation, and adapting meshing. Finally, the modified 2D shell mesh was converted into a 3D solid mesh. Basilar membrane (BM) and Reissner's Membrane (RM) were captured by OCT and recreated in the bony structures to form the three-chamber cochlea model. Cochlear fluid meshes were generated and fluid-structure interaction (FSI) was defined to fully couple the fluid region and solid structures in the FE model.

Results: The FE model of cochlea was successfully reconstructed from the μ CT scans. The 3D fluid-solid coupled mesh of the cochlear bony structures, soft tissues, and cochlear fluid was generated with reasonable element quality and resolution. This model was prepared as an acoustic model to obtain biomechanical responses for future investigation of cochlear mechanism. The results were used to compare with simplified cochlear geometry in previous FE model.

Conclusions: By converting the μ CT-derived 3D model of the cochlea into a FE model, the anatomical accuracy of the human ear representation is significantly enhanced. Compared with previous work, the FE model enable more precise biomechanical analyses and improving its utility in understanding ear injury mechanisms under blast exposure. In addition, the routine for bridging the gap between the 3D reconstructed model from μ CT scan and FE models can be used in future projects.

M132. Towards Atraumatic Inner Ear Diagnostics and Therapy: Development of a Robotic Oct-Guided Insertion Platform

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Background: Over the past decade, Optical Coherence Tomography (OCT) has gained traction as a high-resolution imaging tool for the mammalian and human cochlea. It has enabled the identification and modeling of two critical trauma sites during cochlear implantation: the osseous spiral lamina at the scala tympani entry and the spiral ligament in the first cochlear turn. These observations have been corroborated by contrast-enhanced computed tomography (CECT). Our current research aims to advance a high-precision, multi-sensory robotic system for safe intracochlear insertions, integrating a steerable, miniaturized OCT probe. The broader goal is to enable in situ optical biopsy and develop an atraumatic robotic insertion platform for future inner ear diagnostics and therapy.

Methods: The robotic system comprises two integrated levels. The first is a macro-movement platform (Ref 1), featuring an alignment stage and remote center of motion mechanism aiming to set and manipulate the OCT probe insertion axis. The second is a micro-movement end effector: a piezo-driven, submicrometer-precise, patent-pending concentric tube robot (CTR). The CTR drives the custom, steerable scanning OCT probe to navigate the cochlea up to 270°, overcoming the 2-mm penetration limit of conventional transmembrane OCT (Ref 2). Validation experiments included robotic-assisted insertions in 3D-printed cochlear models and freshly isolated human cochleae.

Results: Real-time visualization of fine intracochlear microstructures, including the scala media, was demonstrated—marking a first in guided probe steering inside the human scala tympani. Current experiments are being conducted with the smaller steerable probe in very fresh specimens with updated OCT technology enabling insertions up to 270°. Previous calibrations have been performed on 3D-printed cochlear model.

Conclusions: Our results demonstrate the feasibility of robotic OCT-guided insertion for atraumatic cochlear navigation and high-resolution imaging. Real-time steering and visualization of delicate intracochlear structures mark an important step toward safe inner ear diagnostics. Ongoing work focuses on probe miniaturization and expanded testing in cadaveric temporal bones to better replicate surgical conditions and support clinical translation.

M133. Artemisinin Derivatives in HEI-OC1 Cells for Potential Antifibrotic Therapy

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Background: Cochlear implant insertion trauma triggers inflammatory processes that can lead to progressive cochlear fibrosis and compromise long-term implant performance. Recent studies reported that artemisinin derivatives, originally developed as anti-malarial agents, possess anti-fibrotic properties. The aim of this work is to characterize their tolerability in inner ear-specific cells to identify potential candidates suitable for local drug delivery systems aimed at preventing cochlear fibrosis.

Methods: We investigated artemisinin, artesunate, dihydroartemisinin (DHA), and artemisone in the murine inner ear cell line HEI-OC1. After 24 h, and 48 h cell viability and cytotoxicity were assessed using luminescence-based MTS and LDH assay, further validated by cell counting via fluorescence microscopy and Hoechst staining. Cell proliferation and adhesion were continuously monitored by real-time cell analysis (RTCA) over 72 h.

Results: MTS, Hoechst, RTCA, and LDH assay confirmed substance- and concentration-dependent effects. Artemisinin did not markedly affect HEI-OC1 viability and proliferation up to 10 μM , with reductions observed only at high concentrations ($\geq 200 \mu\text{M}$). Artesunate and DHA decreased viability and proliferation at mid concentrations (5–10 μM), while low concentrations ($\leq 1 \mu\text{M}$) remained non-toxic. Artemisone reduced cell growth gradually at concentrations from 0.05 to 10 μM .

Contrary to increased cytotoxicity at higher concentrations after 24 h measured by LDH assay and RTCA analysis, a slight decrease was observed at 48 h.

Conclusions: All four substances affected HEI-OC1 cells in a concentration- and time-dependent manner, with artemisinin showing by far the highest tolerability. Dynamics in cytotoxicity after 48 h might indicate adaptive metabolic mechanisms. Identifying non-toxic concentration ranges in this in vitro model provides a basis for subsequent studies on fibroblasts to evaluate antifibrotic potential and support the development of local drug delivery strategies to prevent fibrosis after cochlear implantation.

M134. Effects of Intratympanic Administration of Glucocorticoids on the Guinea Pig Inner Ear

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Background: Recently, we reported the effects of systemic administration of glucocorticoids on the cochlea of guinea pigs. In the steroid groups, expansion of Reissner's membrane and dilation of the endolymphatic space were observed. Clinically, many practitioners have empirically used steroids for the treatment of endolymphatic hydrops-related diseases and sudden deafness, both systemically and locally. This study investigates the effects of intratympanic glucocorticoid administration on the inner ear.

Methods: Fifteen Hartley guinea pigs were used and divided into five groups: dexamethasone-treated (n=5), contralateral dexamethasone-treated (n=5), hydrocortisone-treated (n=5), contralateral hydrocortisone-treated (n=5), and control (n=5). In the control group, 1 ml of saline was injected into the right tympanic cavity via a ventral approach. In the dexamethasone-treated

group, 1 ml of dexamethasone at a concentration of 3.3 mg/ml was injected into the right tympanic cavity using the same method, while the left ear of the dexamethasone-treated group remained intact. In the hydrocortisone-treated group, 1 ml of hydrocortisone at a concentration of 50 mg/ml was injected into the right tympanic cavity using the same technique, with the left ear of the hydrocortisone-treated group also remaining intact.

To assess the effects of glucocorticoids on the inner ear, we measured a cross-sectional area analysis of the scala media at three hours post-corticosteroids treatment using optical coherence tomography (OCT). The degree of a cross-sectional area change was quantitatively measured using a video digitizer.

Results: Ears treated with either dexamethasone or hydrocortisone showed extension of Reissner's membrane and endolymphatic hydrops. Statistical analysis indicated that a cross-sectional area of the scala media increased significantly compared to the contralateral hydrocortisone-treated and control groups.

Conclusions: This is the first report to investigate the effects of intratympanic administration of glucocorticoids on the guinea pig cochlea. Dilated endolymphatic space was observed in the steroid-treated ears. Although the underlying mechanism of these histological changes remains unclear, great care must be taken when administering steroids to patients with endolymphatic hydrops-related diseases, such as Meniere's disease.

M135. The TECTB-C225Y Variant Causing Autosomal Dominant Deafness in a Nicaraguan Family Enhances Sensitivity to Noise-Induced Hearing Loss in Mice

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Background: The tectorial membrane (TM) is an extracellular matrix that lies above the mechanosensitive hair cells in the cochlea. Shearing Motion of the TM plays a major role in displacing stereocilia and facilitating hair cell mechanotransduction. TECTB encodes a major non-collagenous protein of the TM. Here, we discuss a TECTB-C225Y substitution that segregates with autosomal-dominant hearing loss in a Nicaraguan family, and the Tectb-C225Y mouse that models the genotype observed in patients to investigate the variant's effects on

hearing. We carried out a longitudinal study of mouse hearing, a histological assessment of the effect of TECTB-C225Y upon TM structure, and noise exposure tests to determine robustness of the membrane under stress.

Methods: Exome sequencing, linkage analysis and audiometry assessments were carried out for individuals from a Nicaraguan family with autosomal-dominant non-syndromic hearing loss. The hearing of TectbC225Y/C225Y, TectbC225Y/+, and wild-type mice on a corrected Cdh23AHL+ background were tested by ABR and DPOAE at 5 weeks, 3, 6, 9, 12, and 18 months. Samples were collected at 2, 6, and 14 months for histology. TM structure was assessed in histological sections stained with Toluidine Blue. For noise exposure, TectbC225Y/+ and wild-type mice underwent auditory testing before exposure to 100 dB SPL noise for 2 hours at 16 weeks or 18 months of age, and subsequently at 2 days and 2 weeks after noise exposure.

Results: The TECTB-C225Y variant segregated in individuals with mild-to-severe sensorineural hearing loss. In TectbC225Y/C225Y mice, auditory testing showed significantly elevated thresholds in at all time points. Auditory thresholds in TectbC225Y/+ and wild-type mice were not significantly different at any tested time point. Histological analysis showed disruption to TM morphology in TectbC225Y/C225Y TMs at all ages; however, TectbC225Y/+ TMs showed little change in morphology and toluidine blue stain density comparable to that of wild-type at many cochlear locations. Following noise exposure at either 16 weeks or 18 months, TectbC225Y/+ mice developed a greater temporary threshold shift and a much larger permanent threshold shift in response to noise exposure than did wild-type littermate controls.

Conclusions: We propose TECTB as an autosomal-dominant non-syndromic hearing loss gene. We demonstrate that the TECTB-C225Y variant disrupts TM structure and function. Although auditory deficits exhibit recessive inheritance in TECTB-C225Y mice, TectbC225Y/+ mice are more susceptible to noise damage than wildtype mice, mirroring the dominant inheritance identified in the human pathology. These results provide new insight into the role of TECTB in the TM and human hearing loss.

M136. Dynamic Synchrotron-Based Microtomography of Human Tympanic Membrane Motions

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Background: Understanding middle ear biomechanics is crucial for clarifying sound transmission and enhancing surgical techniques. When investigating the tympanic membrane, traditional structural methods like conventional microtomography cannot adequately capture soft tissue dynamics with sufficient resolution. Functional methods like Laser-Doppler vibrometry (LDV) or optical coherence tomography (OCT) offer sensitive measurements, which are however still subject to limitations. We recently explored dynamic synchrotron-based X-ray phase-contrast microtomography to investigate ossicular chain mobility using rigid body transformation. Here we aim to apply this methodology to describe the soft tissue biomechanics of the tympanic membrane.

Methods: We used dynamic synchrotron-based X-ray phase-contrast microtomography on three fresh-frozen human temporal bones under acoustic stimulation at 128, 256, and 512 Hz with sound pressure levels of 120, 126, 141, and 151 dB. Using the recorded stimulation signal from the sine wave generator and the camera exposure trigger, the projection data were sorted into ten phases. Each phase consisted of approximately 4000 to 8000 projections distributed over the 360° rotation

of the sample. Tomographic reconstruction relied on a filtered backprojection with a Fourier-space regridding algorithm. This method achieved micrometer spatial and ~1 kHz temporal resolution, allowing 4D visualization of the ossicles and tympanic membrane. Non-rigid B-spline registration, optimized with anatomical landmarks, provided precise quantification at sub-voxel scales.

Results: The results revealed consistent displacement hotspots in the posterosuperior quadrant of the tympanic membrane and the pars flaccida, although their relative contributions varied between specimens. Fresh1 showed the smallest overall displacements ($< 0.15 \mu\text{m}/\text{Pa}$), Fresh8 the largest (approaching $2 \mu\text{m}/\text{Pa}$), and Fresh7 an intermediate response with a dominant hotspot in the pars flaccida. The overall displacement magnitude decreased as frequency increased, though the

pattern of motion remained similar. The median displacement magnitude (99th percentile) was 3.5 (15.2), 2.4 (11.8), and $2.1 \mu\text{m}$ ($8.3 \mu\text{m}$) for stimulation frequency of 128, 256, and 512 Hz, respectively. The magnitude of displacement changed with the level and frequency of the sound pressure, while the motion patterns remained stable.

Conclusions: Dynamic synchrotron-based X-ray phase-contrast microtomography enables high-resolution, time-resolved imaging of the tympanic membrane allowing quantitative investigation and characterization of its complex motion patterns. We found that TM motion is strongly dependent on frequency and sound pressure level. Displacements were in general highest in the posterosuperior quadrant ranging from 2 - $60 \mu\text{m}$. The pars flaccida also emerged as a consistently mobile region, reflecting its

histological features. Landmark-constrained nonrigid registration allowed accurate quantification of tympanic membrane motion. In general, this method provides unique insight into middle ear biomechanics and opens new possibilities for increasingly precise analyses that incorporate the structure in its full volumetric context.

M137. Synchrotron-Based X-Ray Phase-Contrast Microtomography of Healthy, Sculpted and Pathological Human Auditory Ossicles

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Background: The transmission of sound through the human middle ear is a complex process relying on air-fluid impedance matching and amplification of sound waves by the three smallest bones in the human body: the malleus, incus, and stapes. Their biomechanical properties are linked to their structure, spanning from the molecular (collagen triple helix) to the millimetric scale (e.g., mineral density distribution).

In cases of conductive hearing loss caused by an impaired ossicular chain, reconstructive surgeries are necessary to restore hearing function. In most cases, these procedures involve removing the origin of inflammation and the eroded ossicles followed by ossiculoplasty. The effects of inflammation and the sculpting process on the internal property of the ossicles – such as mineral density, porosity, lacuna distribution, or vascularization – remain poorly investigated. Our goal is to characterize them, to determine whether the sculpting process remains viable over time and whether erosive pathologies alter the in-depth organization, potentially requiring the ossicle's removal and making it unsuitable for use as an autologous implant.

Methods: Our study includes 11 fresh-frozen human auditory ossicles (6 incudes and 5 mallei) in various conditions: 3 healthy, 2 sculpted during ossiculoplasty, and 6 pathological with cholesteatoma, obtained from consenting patients. Synchrotron-based X-ray phase-contrast microtomography was used to obtain high-resolution and high-contrast 3D images of each complete sample. The experiments were performed at the TOMCAT beamline of the SLS (Swiss Light Source, PSI), using a 21 keV beam and a voxel size of 0.65 μm .

Results: For each sample, the relative mineral density distribution was estimated by analyzing the histogram of gray values. In most cholesteatoma cases, the distribution was broader, with a noticeable “low mineral density shoulder”. Four quantitative parameters were extracted from the histograms, and six mineralization classes were defined to generate 3D visualization maps of mineral density. Global porosity indicators, such as vessel density and lacuna density, were calculated using segmented masks of vessels and lacunae. Local porosity was also assessed through thickness maps. Additionally, the variations in local mineralization and local porosity were quantified as a function of the distances to the ossicle's edge and to the nearest vessel. For most evaluated parameters, the cholesteatoma cases showed clear deviation from the healthy ones. In the sculpted cases, the results correlated with how long the ossicles remained in the patient after surgery.

Conclusions: We compared microtomography analyses of healthy ossicles with sculpted ones and those affected by inflammation and erosion due to cholesteatoma. Various parameters related to mineral density, bone porosity and their localization in the ossicle were computed and demonstrated an impact of cholesteatoma on the in-depth bone organization. These findings could provide valuable insights for optimizing middle-ear surgery, with wide-ranging benefits for patients with conductive hearing loss.

M138. Features of the Wideband Acoustic Reflex in Response to Contralateral Noise Elicitors

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Background: The bilateral middle ear acoustic reflex (MEMR) attenuates sound transmission through the middle ear involving a short brainstem feedback pathway primarily controlling the stapedius muscle in humans. We report here differences in the growth of the strength of the MEMR when using contralateral broadband noise (BBN) vs 1/3 octave band noise (OBN). We also noted occasional absorbance change spectra that deviated from repeated measurements with the same elicitor level that did not appear to be caused by random or subject-initiated noise and ask whether these might indicate infrequent activation of the tensor tympani muscle (TT).

Methods: Our measuring system used the Etymotic Research ER-10X probe system to measure changes in absorbance by the ear calculated from the pressure response to wideband chirps caused by activating the MEMR with stimuli delivered to the contralateral ear. The elicitor noise was created digitally and was gated and attenuated electronically under software control.

Results: The change in admittance due to the MEMR was measured as a function of the elicitor level. The threshold for activating the reflex was consistently lowest with BBN elicitors. The reflex threshold for 1/3 OBN elicitors centered at 1 and 2 kHz was at a consistently higher elicitor level, but exceeded the magnitude of reflexes activated by BBN when these narrow band elicitors exceeded ~80 dB SPL.

We also noted changes in the absorbance change spectra showing a pronounced peak near 1 kHz that was absent in most repeats of the same elicitor. A similar 1 kHz peak was noted during voluntary contraction of the MEM. Pronounced peaks at and above 1 kHz appeared to be damped by simultaneous presentation of a 90 dB SPL BBN elicitor. The effects of simultaneous activation with both elicitors was additive below 1 kHz, producing greater reductions in absorbance than either elicitor alone.

Conclusions: The BBN elicitor activated the MEMR at the lowest elicitor levels suggesting that to the input to the brainstem reflex pathway originates from a relatively large cochlear region near threshold. The stronger activation of the reflex by the 1 and 2 kHz 1/3 OBN elicitors at higher levels suggests the presence of neural interactions within the reflex pathway. The occasionally observed peaks in the MEMR induced absorbance change near 1 kHz might be explained by unintentional activation of the TT, as large fluctuations in umbo and stapes velocities above 1 kHz with tensor tympani pulls in the temporal bone study of Cho et al (Hearing Res, 2023), that were not seen for stapedius pulls. The interaction between the voluntary and acoustically activated MEMR suggests separate contributions between the TT and stapedius, as the peaks in absorbance at high frequencies from voluntary contractions appear damped by simultaneous acoustic activation of the MEMR.

M139. Insights From Middle-Ear Impedance in Children With Otitis Media

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Background: Wideband acoustic immittance (WAI) has been shown to help diagnose middle-ear pathology. WAI transfer functions include (1) absorbance and (2) middle-ear input impedance (MEII). Recent evidence has suggested that MEII may offer some advantages over absorbance. Our method for MEII estimation (MEZ) may have clinical value as a supplement to absorbance in the assessment of ears with otitis media.

Methods: This study includes 78 WAI measurements in 60 ears of 33 participants: 37 ears diagnosed with otitis media 23 ears with normal hearing. MEZ was estimated for each ear by fitting parameters of an analog circuit model (that include the impedance of the ear canal and the middle ear) for best agreement with individual WAI measurements at ambient pressure (0 daPa), though WAI responses across static pressure were explored. Middle-ear parameters and round-trip travel time through the ear canal were fit as functions of static pressure. Model-based estimates of conductive hearing loss were compared with audiometric thresholds based on linear regression of average absorbance.

Results: All WAI measurements were well fit by the analog circuit model. Model-based estimates of conductive hearing loss had a mean-absolute difference from audiometric thresholds (pure-tone average) that was < the mean-absolute difference for a regression to pure-tone average from average absorbance (3.8 dB versus 4.6 dB). The WAI stimulus consists of a series of clicks lasting approximately 1.5 seconds. The time-course of specific middle-ear model component values appeared to be more reliable when model-fit errors were smaller. In most cases, distance between tympanic membrane (TM) and the probe (derived from the round-trip travel time through the ear canal) decreased as static pressure decreased from about +200 to -300 dPa. Within the subset of 39 WAI measurements with lowest fit error, (1) the average lateral TM displacement was 0.4 mm and (2) the stiffness and damping of the first MEM branch were both significantly less at tympanic peak pressure (TPP) compared to ambient pressure (0 dPa).

Conclusions: MEZ has the potential to enhance clinical interpretation of WAI measurements. Model-based estimates of conductive hearing loss utilizing MEZ may provide more accurate predictions of audiometric threshold than regression from average absorbance.

M140. Evaluation of Growth and Development of the External Auditory Canal Using 624 Temporal Bone Ct Scans for Appropriate Cochlear Gene Delivery

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Background: Clinical trials on cochlear gene therapy for patients with autosomal recessive deafness 9 via transcanal and transmastoid approaches were reported in 2024. Both procedures are clinically feasible for older patients. However, due to the smaller size of the external ear canal (EAC) in very young patients, it remains unclear whether the transcanal endoscopic approach is applicable. Therefore, we aimed to evaluate pediatric EAC anatomy by using temporal bone computed tomography (CT).

Methods: We analyzed 624 temporal bone CT scans from children (aged 0–18 years) obtained at our hospital between 2017 and 2024. Measurements included the full, cartilaginous, and bony lengths of the posterior and inferior external canals, the angle between the inferior EAC wall and tympanic membrane, the diameter of the narrowest point of EAC, and the tympanic membrane (TM) diameter, all assessed using a standardized method. We analyzed these parameters using change-point (CP) analysis to detect potential age-related shifts. If a CP was identified, we applied a linear regression model allowing different slopes before and after the CP; if not, a simple linear regression was used.

Results: The full and bony lengths of the inferior EAC wall, the angle between the inferior EAC wall and tympanic membrane, and the diameter at its narrowest point increased dramatically between 0 and 1 year of age. In contrast, the CP for the length of the bony part of the posterior wall were not identified and increased linearly. The TM diameter remained unchanged throughout childhood.

Conclusions: The feasibility of transcanal endoscopic ear surgery depends on the diameter at the narrowest point of the EAC, the endoscope (> 1.9 mm, currently available) and surgical instrument dimensions, and the length of the bony part of the EAC needed to elevate a

tympanomeatal flap. Based on our findings, a transmastoid microscopic approach should be considered instead of transcanal approach in patients under 1 year of age.

M141. The Neuronal Network of the Human Middle Ear: Insights From Synchrotron X-Ray Phase-Contrast Imaging

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Background: Accurate identification of the nerves and neuronal networks in the middle ear is of high clinical interest in order to understand the middle ear's physiological function and reduce the risk of iatrogenic injury during otologic surgery. These neuronal networks are susceptible to injury due to their location deep in the temporal bone, complex pathways, small size and close proximity to surrounding anatomical structures. For example, taste changes and dry mouth due to injury to the chorda tympani nerve are experienced by 15–22% of patients who underwent middle-ear surgery.

Methods: Ten healthy human temporal bone samples were imaged using synchrotron-based microtomography. Phase-contrast reconstructions from multiple fields of view were stitched together to generate 3D datasets of the entire peripheral auditory system with an isotropic voxel size of 2.75 μm . The datasets were segmented using 3D Slicer, enabling precise measurements of the nerves and of their spatial relationships to surgically relevant anatomical landmarks.

Results: The anatomical pathway of the chorda tympani nerve was examined in detail along its tympanic and mastoid segments. Its well-known proximity to the tympanic membrane and ossicular chain was measured and categorized. Furthermore, the paths of the tympanic and caroticotympanic nerves were mapped and classified based on their distances to the most surgically important structures, including stapedial and tensor tympani muscles, thereby enhancing anatomical precision. These two nerves form the tympanic plexus, which gives rise to the lesser petrosal nerve, supplying parasympathetic innervation to the parotid gland, sensory innervation to the middle ear cavity, and sympathetic innervation to the vascular plexus.

Conclusions: This study demonstrates that synchrotron-based microtomography enables high-resolution 3D anatomical analysis of middle ear nerves and muscle, providing a level of resolution and quantification not achievable with conventional imaging techniques. Since the middle ear is located deep within the skull, this non-destructive approach allows the study of its delicate structures without the need for dissection, thereby preserving them in their most native state. Such ultra-high-definition visualizations clarify the spatial relationships between nerves, muscles, and surgically relevant landmarks, supporting otologic surgeons in accurately identifying critical structures and reducing the risk of iatrogenic injury.

M142. A Tumor-Bearing Mouse Model for Studying Cisplatin-Induced Hearing Loss

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Background: Cisplatin-induced hearing loss is a significant clinical concern for cancer patients receiving cisplatin-based chemotherapy. Although various tumor-bearing mouse models exist for evaluating cancer therapies, no animal model exists that incorporates cisplatin-induced hearing loss in tumor-bearing mice. Here, we aim to integrate our established cisplatin-induced ototoxicity mouse model with a tumor-bearing mouse model to allow us and others to test whether potential protective therapies can mitigate hearing loss while preserving cisplatin's anti-cancer efficacy.

Methods: Baseline auditory testing was conducted on CBA/J x C57BL/6 F1 mice prior to tumor implantation. To establish a tumor-bearing mouse model with a normal, intact immune system, MOC-1 tumor cells (mouse oral squamous cell carcinoma) were subcutaneously injected into the hind flank of adult mice (syngeneic tumor mouse model). Twelve days later, when palpable tumors were detected, mice were administered either saline or cisplatin. Mice receiving cisplatin underwent two treatment cycles. The first cycle consisted of once-daily cisplatin injections (5 mg/kg) for 4 days, followed by a 10-day recovery period. The second cycle followed the same 4-day cisplatin treatment but was followed by a 15-day recovery period. Weight and body conditioning scores were assessed daily, and tumor volume was measured every other day. At the end of treatment, endpoint auditory testing was performed to assess hearing loss. Auditory function was assessed using auditory brainstem response (ABR).

Results: In MOC1 tumor-bearing mice, cisplatin administration led to significant suppression of tumor growth compared to saline-treated controls. By day 45 post-implantation, cisplatin-treated mice exhibited an approximate 61.14% reduction in tumor volume relative to the saline group. These data indicate that cisplatin effectively suppresses MOC1 tumor growth in this mouse model.

We next assessed whether cisplatin treatment induced hearing loss in the MOC1 tumor-bearing mouse model. Cisplatin-treated mice exhibited significantly elevated ABR thresholds across all tested frequencies (8, 11.2, 16, 22.4, 32, and 40 kHz). Importantly, saline-treated mice implanted with MOC-1 tumors maintained normal ABR thresholds, indicating that tumor presence alone does not affect auditory function in mice. These findings indicate significant development of hearing loss following cisplatin treatment in MOC1 tumor-bearing mice.

Conclusions: We have successfully established a tumor-bearing mouse model that also develops hearing loss in response to cisplatin. This model enables us and others to evaluate potential protective therapies in the presence of tumor, reflecting the clinical use of cisplatin, while ensuring that otoprotective therapies do not compromise cisplatin's anti-tumor efficacy. Using this model, we are currently testing whether pexidartinib (PLX3397), the FDA-approved drug previously shown to completely protect against cisplatin-induced hearing loss, affects the therapeutic efficacy of cisplatin.

M143. Decoding Cochlear Pathology From Auditory Evoked Potentials Using Computational Modeling and Machine Learning

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Background: Auditory evoked potentials (AEP) are widely used in clinical audiology but provide limited insight into the specific cochlear mechanisms underlying hearing loss. Outer hair cells (OHC), inner hair cells (IHC), and auditory nerve fibers (ANF) are mechanically and functionally coupled, making it difficult to disentangle their contributions from recorded responses. Computational modeling offers a way to bridge this gap: forward models can map defined cochlear pathologies to predicted AEPs, while machine learning (ML) can be applied in the inverse direction to infer underlying pathology from measured responses. Such an approach could enable individualized diagnostics at the level of specific cochlear cell populations.

Methods: We employed a physiologically detailed auditory nerve (AN) model (Bruce et al., 2018; Zilany-Bruce-Carney labs) to simulate AEPs across diverse cochlear damage profiles derived from standard audiograms (Bisgaard et al., 2010; Parthasarathy et al., 2020) and aging data (Wu et al., 2021). Each profile was represented as an 18-dimensional label vector, encoding OHC, IHC, and ANF integrity across six cochlear segments with five discrete levels of damage. For each impaired “ear”, responses were generated with 20 individualized “head” models to capture anatomical variability. Stimuli included clicks, chirps, tones, and amplitude-modulated signals across a range of levels and rates. In total, the dataset comprised ~4800 simulated “ear-heads.” Two ML strategies were tested: (1) waveform-based models (CNN + transformer) directly classifying AEP waveforms into multilabel outputs; and (2) feature-based models using physiologically informed descriptors (peak latencies, growth slopes, spectral features) combined with classifiers such as random forests and logistic regression.

Results: Preliminary analyses demonstrate that the framework captures expected physiological dissociations: OHC loss elevates thresholds but preserves suprathreshold amplitudes, IHC dysfunction reduces amplitude growth more uniformly, and ANF loss decreases high-level responses without affecting thresholds. These patterns are consistent with animal (Hirose and Sato, 2011; Furman et al., 2013) and human data (Märcher-Rørsted et al., 2022). Classification performance was robust in simplified tasks (binary healthy vs. impaired; multiclass OHC/IHC/ANF), and initial multilabel classification at cochlear-segment resolution shows promising accuracy trends. Ongoing work extends training to the full multilabel dataset and refines model architectures.

Conclusions: We present a computational/ML framework for mapping AEPs onto cochlear pathology profiles. By leveraging physiologically validated simulations, this approach provides labeled training data unavailable from human recordings, enabling ML models to identify pathology-specific signatures. Results highlight the feasibility of distinguishing OHC, IHC, and ANF dysfunction from non-invasive responses. This paradigm could advance clinical diagnostics toward precision audiology, supporting individualized rehabilitation strategies and future pathology-targeted therapies.

M144. Emergence of Tonotopically Organized Spontaneous Activity in the Brain After Genetic Disruption of Met Channel Function

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Background: Hearing loss is pervasive and associated with other hearing-related disorders such as hyperacusis and tinnitus, and can precipitate cognitive decline and dementia. In tinnitus, a rise in the rate of spontaneous activity is thought to underlie the perception of phantom sounds and appears to be generated in the brain, but we have a limited understanding of how this activity is coordinated or how it propagates through connected brain circuits that used to process sound.

Methods: To determine how hearing loss changes neural activity patterns in the brain, we developed an inducible model of complete hearing loss by deleting *Tmie*, which encodes a MET-associated protein critical for hair cell mechanoelectrical transduction, in 4–8 weeks-old *Gf1l-P2A-GFP-CreERT2;Tmie^{fl/fl}* mice (*Tmie* cKO mice). We expressed genetically encoded Ca^{2+} indicators in these mice and defined the spatiotemporal response characteristics of neurons in the inferior colliculus (IC) and auditory cortex (AC). Spontaneous and tone-evoked neural responses were recorded using wide-field single-photon (1P) and high-resolution, two-photon (2P) microscopy acutely in the IC in awake mice, and longitudinally through implanted cranial windows in the AC, starting during normal hearing and progressing through *Tmie* deletion.

Results: Genetic inactivation of MET function resulted in complete loss of sound-evoked responses within seven days. This loss of sound sensitivity was accompanied by the emergence of large-scale, coordinated sound-independent (SI) neural activity in both the IC and AC. Remarkably, these SI events aligned with the same isofrequency domains evoked by pure tones in control (hearing) mice across lamina in the IC and subdomains of the AC. Two-photon imaging revealed that large-scale coordinated events in *Tmie* cKO mice reflected simultaneous firing of groups of adjacent neurons, which were distinct from the sparse and uncorrelated spontaneously firing of hyperactive neurons that also emerge after deafening. SI activity in the IC of *Tmie* cKO mice was independent of the cochlea, suggesting these tonotopic-like activity patterns are generated within the brainstem. Highly correlated SI activity was also observed in two additional models of hearing loss (cochlear ablation in wild-type mice and *Tmie* inactivation only in outer hair cells), suggesting that this phenomenon is a general feature arising from impaired acoustic input.

Conclusions: Here, we describe a new model of deafness achieved through inducible, genetic inactivation of the MET channel-associated protein TMIE, providing reproducible, temporal control of cochlear inactivation. MET inactivation in hearing mice led to the emergence of correlated neuronal firing aligned to isofrequency domains, resembling sound-evoked response patterns in two brain regions, suggesting that spontaneously generated sound-like patterns are coordinated and propagate through the auditory system after hearing loss. The reproducible nature of this phenomenon holds great promise for uncovering the circuit-level changes responsible for the induction of SI activity following hearing loss that may be perceived as sound.

M145. Distinct Dopaminergic Modulation of Noise-Induced Plasticity With Divergent Effects of Dopamine Receptor Antagonism on Startle and Gap Inhibition

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Background: Noise-induced hearing loss (NIHL) variability among individuals exposed to similar conditions may reflect differential neuromodulatory responses. While synchronous neuronal activity supports auditory precision, asynchronous patterns drive maladaptive plasticity. Dopamine (DA) signaling in the inferior colliculus (IC) represents a critical neuromodulatory pathway, with D1 and D2 receptors mediating distinct cellular responses. This study investigated how D1 versus D2 receptor signaling in the IC contributes to auditory outcomes following temporary threshold shift (TTS) and permanent threshold shift (PTS) noise exposure. This study investigated how DA receptors contribute to startle response alterations following NIHL, specifically examining why total startle magnitude increases while gap inhibition remains intact.

Methods: Male Sprague Dawley rats (n=3-5/group) received bilateral IC cannula implantation and were assigned to saline control, D1 antagonist (R(+)-SCH-23390), or D2 antagonist (S(-)-Eticlopride) treatment groups. Animals underwent TTS noise exposure (16 kHz, 106 dB SPL, 1 hour) following drug infusion. A separate cohort received PTS noise exposure (10 kHz, 118 dB SPL, 1/3 octave 4 hours). Auditory brainstem responses (ABRs) assessed threshold shifts and neural synchrony. Western blotting and immunohistochemistry quantified and localized DA receptor changes. Acoustic startle reflex (ASR) and gap inhibition testing evaluated behavioral responses over six weeks, with pre-pulse inhibition confirming tone detection capabilities.

Results: Western blots confirmed D1 and D2 receptor presence in the IC, with significantly decreased D1 levels following PTS exposure. During TTS, saline controls showed significant threshold shifts at 20 kHz ($p=0.0001$). D1 antagonism completely abolished threshold shifts ($p > 0.05$), while D2 antagonism provided partial protection. ABR amplitude preservation differed between receptor subtypes, with D2 receptor antagonism maintaining greater synchrony than D1 blockade. PPI testing confirmed intact tone detection across groups ($p < 0.001$), while gap inhibition remained unaffected by antagonism of either receptor. Total startle magnitude, however differed across groups.

Conclusions: The preserved gap inhibition despite altered total startle suggests that IC dopaminergic circuits influence descending startle-generating pathways without disrupting ascending gap detection mechanisms. This dissociation indicates that calcium-dependent DA receptor signaling affects brainstem motor output circuits while sparing cortical auditory processing pathways essential for temporal gap detection. Future studies will examine how specific calcium channel manipulations in IC dopaminergic circuits differentially affect startle generation versus gap inhibition pathways, and whether targeting these distinct calcium channel subtypes can selectively modulate maladaptive startle hypersensitivity while preserving protective auditory temporal processing following NIHL.

M146. Effects of Cochlear Hearing Loss on Natural Soundscapes Perception

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Background: Preliminary findings indicate that people with cochlear hearing loss struggle to distinguish natural soundscapes according to habitat, moment of day, and season, and that this deficit is not only due to elevated absolute thresholds.

Methods: The present psychophysical study aimed to investigate the extent to which and how cochlear damage alters perception of natural soundscapes and the influence of ecological factors

such as habitat, moment of day and precipitation level on soundscape perception. Here, perception was examined through the capacity to analyse scenes into their biological and geophysical sound sources. This was achieved by assessing the ability to report sound-source categories (birds, insects, wind, etc.) and estimate the number of birds for each soundscape for normal-hearing (NH) and hearing-impaired (HI) listeners.

The database was recorded in nine pristine habitats, for four moments of day and two precipitation levels. HI participants were tested with unprocessed soundscapes and without their personal hearing aids. NH participants were tested with soundscapes that were either unprocessed or processed to simulate i) threshold elevation with loudness recruitment, or ii) threshold elevation, loudness recruitment and reduced frequency selectivity. Participants were tested under headphones in a soundproof booth, and the unprocessed sounds were presented diotically at 55 dB(A). Responses were collected via a closed-set categorization task proposing predefined source categories (birds, insects, wind, etc.). When birds were detected, the task turned into a numerosity judgment task in which the participant had to estimate the number of birds within the scene.

Results: The results show that cochlear hearing loss reduces the perceived richness of natural scenes and alters the effects of ecological factors on natural-soundscape perception. Hearing loss also alters the perceived composition of the scene with a greater effect on biological sounds compared to geophysical ones. These consequences of hearing loss mainly result from threshold elevation and loudness recruitment, with an additional contribution of reduced frequency selectivity on the overall number of perceived birds, a pattern consistent with the degradation of spectral cues crucial for grouping/segregation mechanisms.

Conclusions: Altogether, these findings highlight the dramatic consequences of cochlear damage on natural sounds and scene perception. They also encourage applications for hearing-aid strategies that aim to preserve cues essential to natural-scene perception.

M147. Differential Effects of Antibiotics on Mitochondrial Function in Inner-Ear Cells and Human Blood Cells

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Background: Antibiotics remain indispensable for treating infections in critically ill patients, yet they may cause adverse effects such as ototoxicity. One proposed mechanism is the unintended targeting of mitochondrial components in eukaryotic cells, reflecting their evolutionary similarity to bacteria.

Methods: We evaluated the impact of five antibiotic classes (carbapenems, fluoroquinolones, aminoglycosides, glycopeptides, and oxazolidinones) on mitochondrial function in an inner-ear epithelial cell line (HEI-OC1) and in human peripheral blood mononuclear cells (PBMCs). At clinically relevant concentrations, we assessed mitochondrial respiration, ATP levels, mitochondrial membrane potential, and reactive oxygen species (ROS).

Results: In HEI-OC1 cells, most tested antibiotics significantly suppressed mitochondrial respiration in intact cells. This suppression was absent in permeabilized cells with controlled substrate supply, indicating that the site of action lies upstream of the respiratory chain.

Consistently, ATP levels decreased and ROS levels increased in HEI-OC1 cells. In contrast, mitochondrial function in PBMCs remained unaffected under identical conditions.

Conclusions: Our findings demonstrate that HEI-OC1 cells are highly sensitive to multiple antibiotic classes. The mechanism of toxicity involves interference upstream of the respiratory chain, leading to impaired respiration, reduced ATP production, and increased ROS generation. These results highlight a potential mitochondrial basis for antibiotic-induced ototoxicity and provide insight into tissue-specific vulnerability.

M148. Therapeutic Effects of HDAC Inhibitors on the Progression of Hereditary Hearing Loss

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Background: Hereditary hearing loss (HL) accounts for approximately 50% of congenital or childhood-onset cases, and early diagnosis and intervention are crucial for improving clinical outcomes and reducing treatment duration. Recently, histone deacetylase (HDAC) inhibitors have emerged as promising therapeutic candidates for slowing the progression of HL.

Methods: We evaluated the protective effects of HDAC inhibitors in two genetic mouse models of progressive HL. In the first model, KCNQ4 p.W276S mutant mice, which exhibit progressive non-syndromic sensorineural HL due to impaired potassium channel activity, were treated with intraperitoneal valproic acid (VPA, 200 mg/kg/day) from 3 to 6 weeks of age. Auditory brainstem response (ABR) was recorded weekly, and cochlear tissues were analyzed at 6 weeks. In the second model, Alport syndrome (AS) mice harboring type IV collagen mutations affecting the cochlear basement membrane received trichostatin A (TSA, 10 mg/kg/day) from 3 to 7 weeks of age. The effects of HDAC inhibitors on HL, stria vascularis thickening, oxidative stress markers, and inflammatory cytokines were assessed. Additionally, in vitro studies evaluated the molecular effects of VPA and TSA on HDAC activity, protein expression, and oxidative stress signaling pathways.

Results: Untreated KCNQ4 mutant mice exhibited HL onset at 4 weeks and near-complete deafness by 6 weeks. VPA treatment significantly attenuated auditory threshold shifts and preserved outer hair cells. VPA also increased histone H4 acetylation and upregulated survival motor neuron (SMN) mRNA levels in cochlear tissue. In vitro, VPA suppressed HDAC1 activity, increased expression of KCNQ4 and HSP90 β , and enhanced their interaction. In AS mice, TSA treatment restored hearing function and reduced stria vascularis thickening. TSA also decreased inflammatory cytokines in cochlear tissue. In vitro, TSA reduced oxidative stress and inflammatory markers and modulated HO-1/Nrf2 signaling pathways.

Conclusions: These findings suggest that HDAC inhibitors, such as VPA and TSA, hold therapeutic potential to slow the progression of hereditary HL by protecting cochlear structures and modulating oxidative stress and inflammation. This may provide a prolonged therapeutic window for more effective intervention in patients with genetic hearing loss.

M149. Diagnostic and Therapeutic Potential of the Near-Infrared Fluorophore P800SO3 in Otitis Media–Associated Bone Resorption

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Background: Chronic and acute otitis media are frequently associated with inflammatory bone resorption mediated by increased osteoclastic activity, which can result in structural alterations of the temporomastoid bone. Furthermore, it may lead to bone defects after surgical interventions for chronic otitis media. P800SO3 is a bifunctional near-infrared (NIR) contrast agent designed with structure-inherent targeting, enabling selective binding to bone tissue without the need for additional binding ligands. This unique property allows P800SO3 to serve not only as an imaging marker but also as a modulator of bone metabolism.

Methods: To investigate the inhibitory effect of P800SO3 on bone resorption under inflammatory conditions, a series of in vivo experiments were conducted using a mouse model. Mice were intravenously injected via the tail vein with either 200 μ M P800SO3 or saline as a control. The distribution and localization of P800SO3 in bone were monitored using near-infrared (NIR) imaging for up to 24 hours post-injection. An otitis media model was established by injecting 3×10^7 MRSA bacteria into the cochlear bulla to induce localized inflammation. Simultaneously, 3 mg of calvarial bone powder—collected from mice previously injected with either saline or P800SO3 (emitting NIR signals)—was introduced into the same bulla site. Inflammation progression was monitored via endoscopic examination at 3 days and 2 weeks post-injection. At 2 months post-injection, micro-computed tomography (microCT) was performed to quantitatively assess bone resorption in the cochlear bulla. In addition, bulla samples were harvested for further mechanistic studies.

Results: Near-infrared imaging performed within 24 hours after tail vein injection of 200 μ M P800SO3 showed accumulation of the contrast agent in the calvarial bone, indicating effective bone targeting and retention. The NIR signal was confined to skeletal structures, demonstrating that P800SO3 possesses structure-inherent targeting capability without the need for additional ligands. At 2 months post-treatment, micro-computed tomography (microCT) analysis revealed a marked preservation of bony architecture in the cochlear bulla of mice receiving P800SO3-injected bone powder. Quantitative assessment showed a significant reduction in bone resorption volume compared to the saline control group.

Conclusions: These findings demonstrate that P800SO3 not only functions as an effective bone-targeted imaging probe but also confers a protective effect against inflammation-induced bone degradation. The results support its potential dual role as both a diagnostic and therapeutic agent for managing bone loss in inflammatory conditions such as otitis media.

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M150. PredHL: Ai-Powered Platform for Pathogenicity Prediction and Assessment of Hearing Loss Genes

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Background: Accurate interpretation of missense variants in hearing loss genes is a persistent challenge in both research and clinical diagnostics. While general-purpose predictors such as REVEL and *ProteinVariant* have advanced variant interpretation, they lack disease-specific resolution and mechanistic context. Recent advances in structure prediction now make it possible to extract mechanistic features across HL-associated proteins, enabling development of disease-focused, structure-informed prediction tools.

Methods: We developed PredHL, a structure-informed machine learning (ML) platform tailored to hearing loss genes. Features were computed from AlphaFold2-predicted protein structures for > 7,000 curated pathogenic and benign variants from the Deafness Variation Database (DVD), incorporating wild-type/mutant physicochemical, evolutionary, and structural properties. Based on this dataset, we trained three complementary model classes: (i) gene-specific predictors for well-characterized HL genes, (ii) protein-type-specific models (globular, transmembrane, collagen) to capture class-specific constraints, and (iii) a disease-specific model covering the full hearing loss gene set. Model performance was evaluated using cross-validation and independently validated on an external ClinVar dataset.

Results: PredHL identified mechanistic signatures distinguishing pathogenic from benign variants, including destabilization energies ($\Delta\Delta G$), changes in solvent exposure, and enrichment of pathogenic substitutions at conserved and structured residues. Gene-specific models achieved the highest Matthews Correlation Coefficient (MCC) values in high-data genes, while protein-type-specific models captured distinct mechanistic constraints across structural classes. While overall performance was comparable to general-purpose predictors, PredHL provided unique value: in multiple hearing loss-critical genes, it correctly identified pathogenic variants missed by both REVEL and AlphaMissense. The publicly accessible PredHL platform enables clinicians and researchers to query missense variants across 218 HL-associated genes, analyze mechanistic feature profiles, and visualize structural impacts in 3D.

Conclusions: PredHL is an AI-powered, disease-focused platform for missense variant interpretation in hearing loss genes. By combining tailored predictors with structural and evolutionary insights, it provides complementary diagnostic evidence, mechanistic interpretability, and broad gene coverage. This platform advances precision medicine for hereditary hearing loss by supporting more confident classification of rare variants and prioritization for experimental validation.

M151. Inner Ear Drug Delivery Route Selection: A Cross-Species Translation Framework

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Background: The delivery of gene or cell therapy to the inner ear represents a challenge due to the blood-labyrinth barrier, necessitating local delivery approaches, especially for expensive therapy with limited therapeutic range, or, susceptibility to systemic side effects. This is particularly relevant for viral gene transfer and cell-based therapies. Adeno-associated virus (AAV) vectors emerge as a prominent choice for gene therapy due to infection efficiency, low toxicity, sustained gene expression, and cost-effectiveness.

Methods: This study aimed to compare different intracochlear (IC) injection techniques in mice, guinea pigs and mini-Swine. Mice were administered via posterior semicircular canal (PSCC) and round window (RW) injections, while guinea pigs received cochleostomy infusion, RW injection (RWI) or deposit (RWD), due to anatomical differences. For swine, injections into the RWI are already being performed in laboratories and transtympanic injection (TT) is still a relevant approach to evaluate RW membrane diffusion. Auditory function effects of IC administration were evaluated in both species via auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE) measurements. Following AAV delivery, GFP (Green Fluorescent Protein, biological marker to visualize gene expression or protein localization) expression from AAV-mediated cell transfection enabled the identification of transfected cochlear regions.

Results: All injection methods successfully targeted hair cells, although transfection patterns and intensity varied depending on the method and substance used. AAV injection provided efficient transduction via both the round window (RW) and posterior semicircular canal (PSCC) routes in mice. Finally, the impact on auditory function differed between injection methods, with PSCC, intracochlear (IC), and transtympanic (TT) approaches showing less effect on DPOAE amplitudes and ABR thresholds compared to the RW route.

Conclusions: In conclusion, our study highlights the importance of refined intracochlear delivery method in animals according to the delivered product and targeted inner ear disorders. While all methods effectively reached cochlear hair cells, variations in transfection patterns, product concentration at targeted cells, and effects on auditory functions, highlight the need for careful consideration of injection strategies. It is worth mentioning that PSCC and cochleostomy routes, though effective in animals, lack translational relevance to humans. Developing these methods in swine (a large- species) would expand research opportunities while avoiding the use of non-human primates, while fostering more ethical practices. Moving forward, further research into optimizing delivery methods will be essential for advancing therapeutic interventions in auditory disorders.

M152. Development of a Wearable Platform for Physiology-Based Personalized Vagus Nerve Stimulation in Tinnitus

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Background: Tinnitus affects 10-15% of the population and significantly reduces quality of life through sleep disturbances, anxiety, and depression. Recently, paired auditory and electrical trigeminal nerve stimulation has received FDA approval, yet this therapy remains inconsistent (up to 25% do not show improvement). The treatment yielded a THI score improvement 2-3 times > the Minimal Clinically Important Difference; however, no one was cured or improved

enough to reach the lowest category of slight handicap. Noninvasive transcutaneous auricular vagus nerve stimulation (taVNS) has shown similar, less-than-optimal results as trigeminal stimulation but has the added benefit of sympathetic stress reduction. A key barrier to progress with the taVNS technique is an inability to induce consistent and predictable physiologic changes with stimulation, as there is an absence of personalized frameworks that can tune stimulation parameters to an individual's unique physiological state, perceptions, and comfort. To address the need for predictability in response to taVNS, we developed an innovative approach that integrates multimodal physiologic monitoring with real-time stimulation control to enable individualized vagus nerve stimulation protocols for tinnitus treatment.

Methods: Our system collects electroencephalography (single channel, Cz active, M1 reference), functional near-infrared spectroscopy (fNIRS), pupillometry, heart rate, heart rate variability, skin conductance, and gastric slow wave activity to capture neural and psychologic responses that can be modulated by taVNS. Utilizing augmented-reality-prompted surveys alongside stimulation, the system integrates measures of personal comfort and percepts of the stimulus as feedback for stimulus waveform optimization. Auditory input is standardized through calibrated in-ear headphones ensuring compatibility with stimulators on the cyma concha. A parameter personalization algorithm was created using a multiobjective optimization framework with Gaussian Process Regression and Pareto analysis to uniquely optimize based on an individual's recorded physiology changes and subjective input to stimulation.

Results: Within MATLAB R2025a, our integrated workflow successfully temporally synchronizes diverse data streams including EEG, autonomic physiology, pupillometry, and survey inputs using Unix and trigger timestamps (EEG/EKG/GSR 10kHz, fNIRS 100Hz, pupillometry 200Hz). Augmented reality surveys integrated with Python 3.10 are effectively answered with joysticks. The parameter personalization was tested with simulated data streams from the recording equipment, able to choose a single optimal parameter from the Pareto solutions.

Conclusions: We present a comprehensive and adaptable test platform for physiology-based personalization of vagus nerve stimulation for tinnitus. By uniting EEG, fNIRS, autonomic physiology, and immersive behavioral assessment with a parameter selection algorithm, the platform enables unprecedented resolution in measuring individual responses to taVNS. This approach provides a foundation for future studies that seek to optimize neuromodulation through biofeedback-based control systems, with the ultimate goal of implementing a precision-medicine approach for the treatment of tinnitus. Ongoing work is in progress to extend testing to a cohort of human subjects and to support translation into clinical otolaryngology.

M153. Grating-Interferometry CT of the Human Ear

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Background: Conventional CT imaging of the temporal bone is limited by insufficient spatial resolution and poor soft-tissue contrast, which hinder detailed visualization of middle and inner ear structures. To ensure accurate diagnosis, an increase in resolution is essential. Improved imaging would also enhance patient counseling, surgical planning, and treatment precision.

Additionally, soft-tissue lesions within the middle ear cavity are particularly challenging to diagnose with current methods, highlighting the need for better soft-tissue contrast in a practical clinical setting. Grating-Interferometry CT (GI-CT) offers a promising solution by combining attenuation, refraction, and scattering signals simultaneously, potentially overcoming these limitations.

Methods: Ten cadaveric temporal bones were imaged using a GI-CT system that features a rotating gantry holding the X-ray source, photon-counting detector, and a three-grating interferometer. Sinograms were generated for attenuation (offset ratios), refraction (phase shifts), and scattering (amplitude ratios). A fused dataset was then created by applying a linear combination of these three contrast channels. Segmentation of bone and soft-tissue structures was performed with 3D Slicer, using both the attenuation volumes and the fused dataset.

Results: Bony structures were depicted with comparable quality in both attenuation and refraction channels, whereas soft tissues were much better visualized in refraction and scattering. Importantly, in the fused GI-CT datasets, soft-tissue structures that are not discernible on conventional CT, such as the chorda tympani, basilar membrane, cochlear and vestibular nerves, and the epitympanic diaphragm, could be clearly identified. Moreover, when comparing fused GI-CT datasets with attenuation-only datasets, the fused images consistently enabled reliable segmentation of soft-tissue elements that were not accessible with attenuation alone.

Conclusions: We demonstrated that the refraction and scattering signals from GI-CT offer additional structural details of the human ear beyond just attenuation. These results serve as proof of concept for using GI-CT in temporal bone imaging and emphasize its potential to enhance clinical diagnostics and support surgical decisions in otology.

M154. Behavioral Timescale Synaptic Plasticity in the Mouse Auditory Cortex

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Background: Learning at the behavioral level can sometimes be ‘one-shot’ or almost instantaneous, but most studies of long-term plasticity such as long-term potentiation (LTP) seem to require dozens or hundreds of induction events yet measure outcomes minutes afterwards. However, recent studies of behavioral timescale synaptic plasticity (BTSP) show that enduring hippocampal plasticity can be induced in just a few trials in vivo and in vitro (Bittner et al. Nat Neurosci 2015, Bittner et al. Science 2017, Liao and Losonczy Annu Rev Neurosci 2024). Here we examine the potential for BTSP to occur in the mouse auditory cortex.

Methods: For in vivo experiments, adult mice were head-fixed and trained on a 2-alternative forced-choice task to distinguish pure tones of one frequency (e.g., 13 kHz) from tones of other nearby frequencies (Martin et al. Nat Neurosci 2024). We monitored calcium transients in A1 pyramidal neurons during behavior. We also examined BTSP in brain slices of adult mouse auditory cortex. We made whole-cell recordings from layer 5 pyramidal neurons in current-clamp mode, monitoring EPSP amplitude and slope before and after pairing events with strong postsynaptic depolarization ~500 ms on 2-10 pairing trials.

Results: We performed 2-photon imaging during behavior and observed sudden, sometimes trial-to-trial changes in frequency tuning curves. For some responsive cells, new peaks to behaviorally relevant stimuli could emerge or be suppressed, shifting overall tuning curve structure. In some cases, unresponsive cells suddenly became responsive to tones during behavioral training. Many of these sudden and enduring changes happened during the earliest phases of training. In brain slice experiments, ~25% of recordings showed BTSP post-pairing when inhibition was intact; this increased to ~50% of recordings with inhibition blocked. This also led to a change in spike output probability after pairing- 3.8% of trials before pairing evoked 1 or more postsynaptic spikes that increased to 27.3% of trials after pairing ($p=0.02$ in paired t-test, $n=6$ cells), without significant changes to resting membrane potential or input resistance. Finally, we pharmacologically blocked L-type calcium channels (LTCCs) and observed no increase in EPSP magnitude or spike probability following pairing protocol ($p=0.18$, $n=2$ cells). This is also observed with hippocampal BTSP (Bittner et al. Science 2017), and we have determined that LTCCs are required for cortical BTSP induction.

Conclusions: We've observed BTSP-like plasticity in the primary auditory cortex with similar molecular mediators as hippocampal BTSP. This suggests a role for behaviorally relevant stimuli to influence changes in synaptic weight across longer timescales in A1.

M155. Orbitofrontal Cortex Transmits Acoustic and Learning-Related Signals to the Auditory Cortex During Perceptual Training

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Background: When it comes to learning motor skills, such as dancing or sewing, “practice makes perfect.” Less appreciated is that our senses also benefit from practice. With training, we can improve our ability to see, hear, smell, and taste – a process termed perceptual learning. In the auditory system, perceptual learning underlies key elements of human social communication, such as speech phoneme discrimination and musicality. Understanding its neural basis could accelerate the acquisition of these skills.

Auditory cortical neurons improve their sensitivity to trained stimuli in parallel with behavioral gains, suggesting that cortical plasticity underlies perceptual learning. Non-sensory processes, like attention or reward, strongly shape auditory cortical responses, and our earlier work showed that this influence grows with perceptual learning. These findings suggest that the networks transmitting non-sensory signals to auditory cortex are themselves plastic. Yet the circuits and mechanisms involved remain unclear. We recently showed that inactivation of the orbitofrontal cortex (OFC) disrupts task-dependent auditory cortical plasticity and sound perception. Here we asked how training impacts the activity of OFC neurons, hypothesizing that OFC signals to the auditory cortex strengthen during perceptual learning.

Methods: We first asked whether auditory perceptual learning is associated with changes in OFC neuron firing properties. To answer this question, we recorded extracellular activity from OFC neurons in freely moving Mongolian gerbils as they trained to detect progressively weaker amplitude modulated stimuli. To test whether learning-related changes occur in OFC neurons that innervate the primary auditory cortex, we used fiber photometry to record calcium signals

from both their cell bodies in OFC and from their axonal terminals in the deep layers of auditory cortex.

Results: Extracellular recordings revealed two populations of OFC neurons: one whose firing scaled with AM depth, and another whose firing was insensitive to AM depth. In this latter group, tonic activity gradually increased over the course of perceptual learning and predicted behavioral improvement. Fiber photometry of OFC cell bodies projecting to auditory cortex revealed trial-evoked calcium transients with two components: an early response that scaled with AM depth, and a later response that increased with learning but was independent of AM depth. In contrast, photometry of OFC axon terminals in auditory cortical layers 5/6 revealed calcium transients whose magnitude scaled both with AM depth and learning.

Conclusions: Our findings indicate that OFC neurons send at least two distinct signals to the auditory cortex: one that scales with AM depth and another that reflects learning. Our electrophysiological recordings suggest that these signals originate from different OFC populations. Together, these results imply that during perceptual learning, the OFC's modulatory influence on auditory cortex strengthens while stable, acoustically-driven feedback is maintained.

M156. Stimulus-Specific Adaptation (SSA) to Pure Tones in Local Networks of Mouse Auditory Cortex

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Background: Stimulus-specific adaptation (SSA) is the reduction in responses to a common stimulus that does not generalize, or only partially generalizes, to other, rare stimuli. In human studies, related brain potentials (such as Mismatch Negativity, MMN) are tested for a stronger property called 'deviance sensitivity'. We test SSA for deviance sensitivity by comparing the responses to a deviant tone in an oddball sequence with two controls: the rare tone presented by itself (deviant-alone), and the rare tone presented as part of a sequence of many widely spaced frequencies (diverse broad). Deviance sensitivity requires that the responses in the deviant condition be larger than in the diverse broad condition.

Using fiber photometry and wide-field imaging of calcium signals in the mouse primary auditory cortex, we uncovered large and robust response components which show large responses to rare sounds, independent of their context (deviant, deviant-alone and diverse broad) and are often larger in the deviant than in the diverse broad condition.

Methods: To study the single-neuron activity that underlies these deviance-sensitive population responses, we used two-photon microscopy. We imaged neurons from upper layer 2/3 of the primary auditory cortex of mice.

Results: Neurons showed in general large responses to the three rare conditions (deviant, deviant-alone and the diverse broad) but formed distinct groups that seemed to prefer either deviant, deviant-alone, or diverse broad presentations of one frequency or the other. In consequence, population patterns distinguished all three rare conditions.

Conclusions: Thus, the deviance of the deviant tones in an oddball sequence is reflected by specific population response patterns, in addition to the size of the responses they evoke.

M157. Hearing in Very Different Human Populations

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Background: To date, there is almost no information available comparing the hearing of different human populations. Most studies were based on Europeans or Asians or their descendants, in e.g., N. America. There is a > 30% variation in the length of human cochleae, so it is probable that this results in measurable differences in auditory performance between individual humans and perhaps populations. With this contribution we propose deeper study of this theme.

The San people of southern Africa represent one of the oldest distinct human populations, being almost completely separate from all other groups for > 100k years. We report the results of a study of 32 young adults (18 to 25 years of age) belonging to the Ju/'huansi of the Nyae Nyae San community based around the town of Tsumkwe in the Otjozondjupa district of Namibia and will compare these to an ongoing study of West European students of Groningen University, the Netherlands, using the same apparatus. The Namibian study was approved by the National Commission on Research, Science and Technology of the Government of Namibia under permit number RPIV022112023 and by the local San community.

Methods: Using a closed insert microphone system, we measured audiogram thresholds in both ears up to 16kHz. In addition, we gathered the spectra of spontaneous emissions (SOAE) and examined SOAE suppression tuning. Stimulus-frequency otoacoustic emission (SFOAE) data are reported in a separate abstract (Maat et al.). Subjects were seated in sound-isolation booths. Daily total measurement times did not exceed 2 hrs.; additional data were collected in subsequent sittings.

Results: Namibian subjects, who are generally smaller in stature than Europeans, produced slightly lower background noise between 1 kHz and 3 kHz (minimum -18 dB SPL). After exclusion of 6 subjects with obvious hearing loss, or measurements in which there were technical problems, mean audiogram thresholds in the two groups were identical. There were, however, substantial differences between the number and levels of SOAE in the two groups. The SOAE peak values were on average higher in Namibian subjects, both in peak frequencies (13.7 kHz in 45 ears of Namibians vs. 4.4 kHz in 16 ears of Europeans) and the highest levels (18.6 vs. 11.1 dB SPL, respectively). Assessment of SOAE tuning is ongoing.

Conclusions: Despite more than 100,000 years of separate evolution, the thresholds of San and European young people were the same. This could mean that both groups maintained the ancient thresholds, or that they simply evolved in parallel. The SOAE of the San subjects clearly had higher mean levels and frequencies. It is possible that this is due to the greater exposure of Europeans to ubiquitous societal noise, which is expected to have had measurable effects on cochlear function.

M158. Open Board

M159. Comparisons of Otoacoustic Emission Between Two Evolutionary Lineages of Homo Sapiens'

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Background: There are very few studies comparing the auditory abilities of different human populations. Most studies of human hearing were based on Europeans or Asians or their descendants, in e.g., N. America. Since the length of human cochleae varies greatly (> 30%), it is likely that there are measurable differences in auditory performance between individual humans and perhaps populations. With this contribution we propose deeper study of this theme.

The San people of southern Africa represent one of the oldest distinct human populations, being almost completely separate from all other groups for > 100k years. We report the results of a study of 32 young adults (18 to 25 years of age) belonging to the Ju/'huansi of the Nyae Nyae San community based around the town of Tsumkwe in the Otjozondjupa district of Namibia and compare these to data from students of Groningen University, the Netherlands. This study was approved by the National Commission on Research, Science and Technology of the Government of Namibia under permit number RPIV022112023 and the local San community.

Methods: Using a closed insert microphone system, we gathered the spectra of spontaneous (SOAE) and stimulus-frequency otoacoustic emissions (SFOAE) and examined SOAE suppression tuning. Data on thresholds in both ears up to 16kHz, and characteristics of the SOAE spectra are reported in a separate abstract (Manley et al.). Subjects were seated in sound-isolation booths and daily measurement sessions were limited to a duration of 2 hrs.; additional data were collected in subsequent sittings.

Results: SFOAE average levels across the whole frequency range were the same in subjects from both groups. A comparison of SFOAE phase revealed, however, that the mean NSFOAE normalized group delays from Namibians were about 10% lower (0.88) than of those of Europeans. If SFOAE delay is directly related to cochlear filter width, the Namibians would have slightly broader frequency tuning curves.

Conclusions: Although there was substantial overlap in the performance of the ears of the two groups, frequency selectivity as indicated by the SFOAE delays was lower in the Namibian population. Since the San are physically smaller than Europeans it is possible that the cochlea is also on average smaller and thus the organ of Corti shorter, potentially affecting frequency selectivity. For future studies, it would be important to compare the cochlear dimensions and auditory performance in different human populations, since this has important implications for surgical interventions and cochlear implantation.

M160. Effects of Averaging on the Reliability of Click-Evoked Otoacoustic Emission Measures of the Medial Olivocochlear Reflex

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Background: The medial olivocochlear reflex (MOCR) can be assessed by measuring the suppression of otoacoustic emissions (OAEs) in the presence of contralateral acoustic stimulation (CAS). However, MOCR is a small effect, highly sensitive to measurement variability and signal-to-noise ratio (SNR). Previous studies have used diverse and often low SNR criteria, raising concerns about the reliability of published findings. This study aimed to determine the optimal SNR threshold for reliable MOCR assessment and to compare measurement protocols.

Methods: Click-evoked otoacoustic emissions (CEOAEs) were recorded in 24 normally hearing adults using 65 dB peSPL clicks, both with and without 60 dB SPL contralateral white noise. Two measurement protocols were compared: interleaved CAS (alternating with/without contralateral noise) and constant CAS (sequential measurements without and with noise). Multiple recordings were obtained per subject, each terminated when predefined SNR criteria were reached (< 3 , > 3 , > 6 , > 6 with noise < 6 , > 12 , and > 20 dB). Each subject underwent two measurement sessions. MOCR magnitude and its repeatability were analyzed across criteria and protocols.

Results: Raising the SNR criterion progressively reduced variability in MOCR measurements. The largest improvement occurred when moving from < 3 dB to > 6 dB, with further benefits observed up to > 12 dB. Beyond 12 dB, gains were modest, and the number of ears meeting the stricter criteria declined markedly. Interleaved CAS provided greater reliability compared with the constant CAS approach. Expressing MOCR in both dB and percentage terms showed similar trends.

Conclusions: For reliable assessment of MOCR using CEOAEs, an SNR criterion of at least 12 dB is recommended. Interleaved CAS protocols further enhance repeatability. These findings suggest that many previous studies employing lower SNR thresholds may have reported less reliable MOCR estimates. Adoption of higher SNR standards could enable broader and more consistent application of MOCR in auditory research and clinical settings.

M161. Large Language Models in Auditory Brainstem Responses Interpretation

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Background: Auditory brainstem responses (ABRs) are a standard objective measure for estimating hearing thresholds, but their interpretation requires expertise and is time-consuming. Recent advances in large language models (LLMs) such as ChatGPT, Gemini, and Claude suggest the potential for automated threshold detection from image data. This study evaluated the performance of three state-of-the-art LLMs in estimating ABR thresholds from printout images compared with human experts.

Methods: A dataset of 110 printout images of click-evoked ABRs, each showing waveforms across stimulus levels, was analyzed. Three LLMs (ChatGPT, Gemini, and Claude) were prompted to detect hearing thresholds directly from the images. Expert clinicians independently reviewed the same images, and their assessments served as the reference standard. Agreement was quantified using correlation coefficients and mean absolute differences in threshold estimates.

Results: All three LLMs demonstrated high correlation with expert judgments ($r > 0.95$). The average difference between AI-derived thresholds and expert ratings ranged from 7.8 to 9.9 dB. Claude showed particular strength in evaluating challenging cases where experts judged no clear ABR responses to be present, outperforming ChatGPT and Gemini in correctly identifying absent responses.

Conclusions: State-of-the-art LLMs can estimate ABR thresholds from clinical printout images with accuracy comparable to expert interpretation. While differences of up to ~10 dB remain clinically relevant, the strong correlations highlight the potential for integrating AI support into audiological diagnostics. Claude's advantage in detecting absent responses suggests differential strengths across models. Further development and training on larger, clinically diverse datasets may enhance performance and support future clinical adoption.

M162. Singe-Sided Deafness: Who Uses What?

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Background: Despite normal hearing in one ear, single-sided deafness (SSD) can profoundly impact patients functionally, socially, and psychologically. As understanding of the impact of SSD has progressed, so have the options that offer various extents of aural rehabilitation – contralateral routing of signal (CROS) hearing aids, osseointegrated devices, cochlear implants (CI), or nothing at all. We seek to better understand the real-world experiences of patients with SSD and the factors influencing the selection of a specific aural rehabilitation device.

Methods: This retrospective, qualitative, and semi-quantitative study gathers patient-reported outcomes using validated survey questionnaires and responses from structured interviews, highlighting device use and the emotional impacts of SSD. Patients who sustained SSD and underwent otologic and audiologic management between 2000 - 2025 were invited for participation. Inclusion criteria involves patients who met cochlear implant candidacy with a PTA of ≤ 30 dB HL in the better hearing ear. The functional and tinnitus impacts of each device are being explored using the Speech, Spatial, and Qualities of Hearing Scale (SSQ12) and Tinnitus Handicap Index (THI). Additionally, demographic data, including ZIP code, education level, and insurance status, are collected from patient charts. The on-going study has recruited cochlear implant (n=5), osseointegrated device (n=1), and CROS hearing aid (n=11) patients, as well as patients with no device (n=8).

Results: Due to an insufficient number of BAHA users recruited, this group was excluded from the initial analysis. 100% of cochlear implant users still wear their device, compared to 82% of CROS users. Baseline data shows that cochlear implant users wear their device an average of 7 days a week, while CROS users wear their device an average of 4 days a week. Cochlear implant users demonstrated the highest total SSQ12 scores (mean = 70), followed by CROS users (mean = 57) and individuals without a device (mean = 52). CROS users demonstrated the best THI score, indicated by a lower score, with a mean of 17, compared to cochlear implant users (mean = 23) and no device (mean = 40). Structured interviews show that CROS patients report dissatisfaction with their device, especially in social settings. Structured interviews further

suggest that device choice is influenced by insurance coverage and perceived invasiveness of surgery.

Conclusions: Real-world patient experience offers important perspectives in optimizing aural rehabilitation in SSD. While the study is ongoing, current data trends suggest that cochlear implant users experience greater device use and perceived benefit. Structured interviews have highlighted the social impact that SSD has on patient lives, emphasizing the need for counseling and support tailored to the challenges these patients face.

M163. The Role of Trefoil Factor Proteins in the Nose of COVID-19 Hamster Anosmia Models

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Background: Trefoil factors (TFFs) are proteins primarily found in the gastrointestinal tract, where they play an important role in mucosal defense and repair. TFF3 is expressed within goblet cells in the small intestine and colon, while TFF2 is expressed in the stomach and duodenum. The nose also contains goblet cells and other mucus-secreting glands, and while TFFs have been found in the nose, their role in nasal tissue repair after infection remains unclear. To investigate this, we examined TFF2 and TFF3 expression in the nose at various stages of healing post SARS-CoV-2 infection.

Methods: Hamsters were intranasally infected with SARS-CoV-2. Olfactory function behavior was tested at various days post-infection (dpi): 2, 3, 5, 8, 17, 21, 35, and 42 dpi, before nasal tissue analysis. Cross-sections of the nose were collected and immunohistochemically labeled with TFF polyclonal antibodies. Labeling characteristics were analyzed in different regions of the nose and submandibular gland, with a focus on epithelial cells, submucosal glands, Bowman's glands, goblet cells, nerve bundles, blood vessels, and the submandibular gland (mucous and serous acini). Universal labeling in the septal cartilage was used as a reference for labeling intensity to compare between hamsters.

Results: Goblet cells were not labeled with TFF3; however, some labeling was observed with TFF2. Similarly, TFF3 labeling was not observed in any epithelium, while TFF2 was, with the brightest olfactory and respiratory epithelial labeling observed at 2 dpi. Both proteins were labeled in Bowman's glands and submucosal ducts. While TFF3 labeling peaked at both 3 and 21 dpi, TFF2 showed an early peak at 3-5 dpi followed by a gradual decline. In the olfactory neuroepithelial region, TFF3 labeling was strongest at 21 dpi, while TFF2 peaked at 2 dpi. In the submandibular gland, both proteins showed parallel trends. Mucous acini were labeled strongly at 5 dpi with another peak at 42 dpi, while serous acini peaked between 17–21 dpi. Compared to TFF3, TFF2 expression was more consistent in submandibular ducts and the vomeronasal organ across all time points.

Conclusions: The lack of TFF3 labeling in goblet cells in the nose suggests these cells serve a different function than those in the GI tract. TFF3 expression in mucus-secreting glands and ducts for long periods suggests that it may act as an inflammatory mediator to SARS-CoV-2 prior to tissue regeneration, while TFF2 plays a role as an inflammatory mediator at early stages of infection. Additionally, the presence of TFFs in neuronal cells, cartilage, and blood vessels

suggests a potential role in SARS-CoV-2 anosmia complications by regulation of the mucus secretory environment in the nose.

M164. Hearing Loss Cohort in Lassa Virus Endemic North Central Nigeria

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Background: Lassa virus (LASV) infection is a major endemic disease in West African countries associated with severe sensorineural hearing loss. Up to 30% of those infected with LASV develop hearing loss. The goal of this study is to characterize the hearing loss associated with LASV infection in North Central Nigeria to elucidate the mechanism leading to hearing loss.

Methods: The subjects were recruited through Jos University Hospital in Nigeria. A cohort study on 1) subjects with LASV infection and 2) subjects with hearing loss with or without LASV infection were carried out.

Subjects were grouped as follows: new onset Lassa fever (LF) patients (n=13), LF survivors with hearing loss (n=11), hearing aid users (n=121), and cochlear implant users (n=4). Detailed history and physical examination, pure tone audiometry, otoacoustic emission, auditory brainstem response, LASV antibody titer, and questionnaire was performed. Pure tone average (0.5Hz + 1kHz + 2kHz + 4kHz)/4 (dB) was calculated using WHO classification and audiogram configuration was reported for the better hearing ear. Of the 169 audiograms reviewed, 149 fit selection criteria and were included in the study.

Results: Most subjects (92.31%) with new onset LF had normal hearing whereas in the LF survivors, nearly half of the subjects had moderate or a more severe degree of hearing loss. In the hearing aid users group, the largest proportion had profound hearing loss (40.5%), 14.88% had severe hearing loss and 14.05% had normal hearing, while 100% of the cochlear implant group had profound hearing loss.

Audiometric configurations varied by group: LF survivors and hearing aid users showed broadly similar patterns, with flat (54.55% vs. 50.41%), sloping (18.18% vs. 25.62%), and tent-shaped (9.09% vs. 5.79%) configurations. In cochlear implant users, the majority was either flat (50%) or sloping (25%) configuration.

Conclusions: The normal hearing in the new onset LF group was consistent with the expectation that it can take weeks or months before developing hearing loss. The cochlear implant group, as expected, had profound hearing loss as the criteria to get an implant.

The LF survivors and hearing aid users groups showed a comparable distribution of hearing loss severity, with a high proportion of profound loss. Both groups also showed similar audiometric configurations, though the higher percentage of sloping configuration in the hearing aid users may be attributed to age differences between the cohorts. These similarities in the audiometric characteristics between the two groups may be suggestive of the influence of LASV infection on the pathophysiology of hearing loss in this region.

M165. A Novel CT "Moth-Eaten Sigmoid Plate Sign" Unveiled: Its Link to Dural Arteriovenous Fistula-Induced Pulsatile Tinnitus

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Background: To highlight that dural arteriovenous fistula (DAVF) can exist in subjects with unilateral vascular pulsatile tinnitus (PT), positive internal jugular vein (IJV) compression tests, and radiologic evidence of sigmoid sinus wall anomalies (SSWA).

Methods: A retrospective analysis was conducted on 80 subjects with PT as the primary symptom and intracranial DAVF detected based on magnetic resonance angiography results. Among them, IJV and retroauricular compression tests and temporal bone computed tomography (CT) were performed. The moth-eaten sigmoid plate sign and DAVF-induced SSWA were defined, and their correlation with PT duration was studied.

Results: Significant differences were observed between ipsilateral IJV and retroauricular compression outcomes in DAVFs located at the transverse-sigmoid sinus ($P < .001$) and hypoglossal canal ($P = .026$). Among 71 subjects with CT data, the moth-eaten sign was found in 29 of 37 subjects (sensitivity 78.4%, 95% CI 0.63-0.89) with DAVFs at the transverse-sigmoid sinus. SSWA and jugular bulb (JB) anomalies were observed in 40.8% of subjects. PT duration significantly differed between subjects with SSWA and those without SSWA ($P < .0026$).

Conclusions: The presence of SSWA on CT and a positive IJV compression test should not be considered conclusive for diagnosing venous PT. The "moth-eaten sigmoid plate" sign on non-contrast CT and positive retroauricular compression are strong indicators of DAVF as the primary cause of PT.

M166. Sigmoid Sinus Wall Anomalies Can Progress and May Not Be Congenital

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Background: Sigmoid sinus wall anomalies (SSWA) are closely linked to venous pulsatile tinnitus (PT). This study aims to demonstrate that SSWA develops progressively rather than being congenital.

Methods: We retrospectively analyzed 42 PT patients with SSWA who had at least two non-operative CT scans at our clinic. CT images were longitudinally assessed to track SSWA progression, while MRI and Doppler ultrasound evaluated transverse sinus stenosis and venous hemodynamics. Changes in PT perception were tracked using the tinnitus handicap inventory (THI) questionnaire.

Results: Among the 42 SSWA patients, 12 (28.6%) exhibited progression. Anastomosis between diploic vein and diverticulum was significantly higher compared to the dehiscence cohort ($p < 0.01$). Within the diverticulum group, seven individuals (30.4%) experienced enlargement, with a

mean diverticular wall expansion of $5.9\% \pm 11.4\%$. Progressive erosion was observed in two cases (12.5%) in the dehiscence cohort, with a mean sigmoid plate erosion of $3.8\% \pm 10.1\%$. In cases progressing from dehiscence to diverticulum, three subjects transitioned, with a mean sigmoid sinus wall length expansion of $43.8\% \pm 31.9\%$. SSWA progression showed a significant negative correlation with QBILATERAL ($r = 0.857$, $p = 0.014$), and there was a significant difference between initial and revisit THI scores ($p < 0.01$).

Conclusions: SSWA can undergo morphological progression, indicating it is a progressive clinical condition rather than congenital.

M167. Hydroacoustic Sonification and Flow Pattern Investigation of Venous Pulsatile Tinnitus Using Mems Hydrophone Sensing and Dye Flow Visualization Techniques

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Background: Although venous pulsatile tinnitus (PT) is surgically treatable, the surgical efficacy has unfortunately remained inconsistent owing to the relatively unknown mechanistic and psychophysical aspects of venous PT. We investigated the connection between the hydroacoustic and psychophysical characteristics of venous PT in this study.

Methods: All 3D-printed models were reconstructed on the basis of two PT patients with sigmoid sinus wall anomalies using their magnetic resonance images. A MEMS hydrophone sensor was placed in the jugular bulb region intraluminally to study the operative alteration of the fluid-borne sound. The dye flow visualization technique used to investigate flow patterns was conducted in this study.

Results: The hydrophone experiment revealed that cases 1 and 2 experienced a significant reduction in the peak amplitude of fluid-borne sound detected by the hydrophone sensor. In case 1, the largest preoperative flow amplitude within the humanly audible frequency range (20 Hz–20 kHz) was 103.5 dB SPL at 98.5 Hz. After the volumetric change and extraluminal compression of the transverse-sigmoid junction, it decreased to 100.4 dB SPL at 20.5 Hz. For case 2, the preoperative peak flow amplitude was 101.6 dB SPL at 46.5 Hz, which reduced to 95.1 dB SPL at 63.7 Hz postoperatively. The estimated mean velocity of the illuminated working flow was 0.208 m/s. Initially, the flow velocity increased as it passed through the stenosis, forming a jet-like flow with a transient velocity estimated to exceed 0.75 m/s. The flow velocity markedly decreased at the transverse-sigmoid junction and jugular bulb, indicated by increased visible rotational flow motion and the development of flow stagnation from the jet flow impacting the transverse-sigmoid junction.

Conclusions: Audio data acquired from the MEMS hydrophone sensor technique indicates that venous PT is the perception of fluid motion. The acoustic profile of venous PT varies, which is associated with the ipsilateral flow volume, flow velocity, and flow pressure gradient.

M168. Multimodal Treatment of Tinnitus in Patients With Comorbid Depression: An Educational and Clinical Approach

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Background: Tinnitus, frequently accompanied by comorbid depression, can severely impair quality of life. Repetitive transcranial magnetic stimulation (rTMS) has emerged as a promising non-invasive treatment for tinnitus, especially when addressing both auditory and emotional networks. However, comprehensive therapeutic approaches, including psychotherapy and pharmacotherapy, are often needed.

Methods: This study evaluates the effectiveness of a combined therapeutic approach, including sequential prefrontal and temporoparietal rTMS, tinnitus psychotherapy, and antidepressant treatment in 20 patients with chronic tinnitus and comorbid depression. Twenty patients diagnosed with chronic tinnitus and comorbid depression underwent a multifaceted treatment regimen that included 10 sessions of sequential rTMS targeting the dorsolateral prefrontal cortex (DLPFC) and the primary auditory cortex. In addition to rTMS, tinnitus psychotherapy and antidepressant medication were administered. MRI and audiometry were performed both before and after treatment to evaluate changes in brain activity and auditory function. Tinnitus severity and treatment efficacy were assessed using the Tinnitus Handicap Inventory (THI), Tinnitus Functional Index (TFI), and additional validated scales for depression and quality of life

Results: Patients demonstrated a significant reduction in tinnitus severity, with an average TFI score improvement of 40% and THI score reduction of 50%. Audiometric evaluations showed stable or improved hearing thresholds, and MRI indicated changes in the targeted brain regions. Comorbid depression symptoms improved modestly, with antidepressant treatment contributing to mood stabilization. The combination of rTMS, psychotherapy, and pharmacotherapy resulted in substantial improvement in both tinnitus perception and emotional well-being. No major adverse effects were observed, although two patients reported mild, transient headaches.

Conclusions: The combined therapeutic approach of rTMS, tinnitus psychotherapy, and antidepressant treatment effectively reduces tinnitus severity and improves associated depressive symptoms. The use of MRI and audiometry provides objective insights into brain and auditory function changes, supporting the clinical improvements observed. This comprehensive treatment approach warrants further investigation for long-term efficacy and optimization.

M169. Tinnitus-Associated Changes in Single Auditory-Nerve Fiber Responses to Tone Bursts

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Background: Peripheral cochlear damage is the main risk factor for tinnitus development, while tinnitus maintenance is strongly correlated to altered activity in the auditory brainstem dorsal cochlear nucleus. Therefore, we aim to investigate the role of the auditory nerve, which connects these two structures, in tinnitus development. Previously, we found that spontaneous activity is

significantly reduced in auditory-nerve fibers (ANFs) in animals with behavioral signs of tinnitus a few days after noise exposure. To identify possible cochlear pathologies associated with this decrease in spontaneous activity, the current study examined standardized metrics from ANF responses to tone bursts that are known to correlate to cochlear functions.

Methods: Anesthetized Mongolian gerbils ($n = 18$) were unilaterally exposed to narrowband noise (115 dB SPL, centered at 4 kHz). In 3 of 18 animals, the noise-exposure speaker was disconnected to create a so-called ‘sham-exposed’ condition. After one recovery day, animals were behaviorally tested for tinnitus using the gap-prepulse inhibition of the acoustic startle reflex (GPIAS) paradigm. Three days after noise exposure, spiking activity from single-unit auditory-nerve fibers was recorded using sharp glass electrodes while presenting tone bursts. Peri-stimulus time histograms to best-frequency tone bursts at 30 dB above threshold were used to determine first-spike latency, jitter, peak- and saturation rates, and fano factors. These metrics are thought to be associated to inner-hair cell and synaptic features, such as pre-synaptic calcium buffering and dendritic efferent innervation. The Q10dB was derived from the tuning curve to determine frequency selectivity, which is closely related to outer-hair cell function.

Results: Average spontaneous rate was significantly lower in ANFs from animals with signs of tinnitus compared to sham-exposed and no-tinnitus animals, confirming previous results. Analyses of the peri-stimulus time histograms showed that the responses from ANFs recorded in animals with signs of tinnitus differed in various aspects to those recorded from animals without signs of tinnitus and from sham-exposed animals. Specifically, animals with signs of tinnitus had significantly longer first-spike latencies, more jitter, lower peak- and saturation firing rates, and higher fano factors. On the other hand, responses from animals without signs of tinnitus did not differ in any aspect to those from sham-exposed animals. Furthermore, fiber selectivity did not significantly differ between ANFs recorded from animals with signs of tinnitus, those without signs of tinnitus, and sham-exposed animals.

Conclusions: These findings reveal that tinnitus development is associated with altered response properties at the level of the auditory nerve, suggesting changes in inner-hair cell and synaptic functioning, but not in outer-hair cell functioning. Ongoing analyses aims to determine how these changes in responses to tone bursts are connected to the tinnitus-associated decrease in spontaneous activity.

M170. Evaluation of Tinnitus and Hyperacusis Using a Custom AI-Powered Pupil Dilation System

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Background: Pupil dilation is an important physiological marker regulated by the autonomic nervous system. Recent studies have shown that pupil dilation is sensitive to sound stimuli, and people with sound-sensitive disorders showed an enlarged response to sound compared to control subjects. These studies suggested that pupil dilation may be used as an objective measure for tinnitus and hyperacusis severity. As the current systems for measuring pupil dilation (e.g., EyeLink) are expensive and not suitable for clinical use, we developed AudioSight, a pupil dilation system that employs an AI-based algorithm to detect changes in pupil size and derive key parameters related to sound-induced emotional responses.

Methods: Our AudioSight systems have both PC version and mobile phone version, designed for clinical office use and patient home use. Using AudioSight, we have tested individuals with tinnitus and hyperacusis and compared their results with control subjects using multiple testing procedures, including the listening effort test, loudness test, and aversive response test.

Results: Our preliminary results have demonstrated that individuals with hyperacusis and tinnitus exhibit pronounced pupil dilation, particularly in the form of peak amplitude and heightened excitatory phases in the listening effort test and aversive response test.

Conclusions: The system represents an advancement in objectively quantifying sound tolerance and arousal responses, offering potential improvements in clinical diagnosis of tinnitus and hyperacusis. By enabling more objective assessments, AudioSight could provide accessible and cost-effective assessments for tinnitus and hyperacusis.

M171. Gap Pre-Pulse Inhibition of the Acoustic Startle in the Objective Assessment of Tinnitus in Humans Using Electroencephalography

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Background: A limitation in current clinical trials on tinnitus is the reliance on subjective self-reported assessments as an outcome measure. Recently, it has been proposed that magnetoencephalography (MEG) recordings in humans of cortical evoked responses to the gap pre-pulse inhibition of the acoustic startle (GPIAS), a paradigm used pre-clinically to objectively assess tinnitus, offer greater sensitivity than the traditional blinking response. Here, we present preliminary results from the Tinnitus DEtection (TIDE) project on a recently completed recruitment of 40 chronic tinnitus cases and controls at the Otolaryngology Head and Neck Surgery Department of the University Hospital in Tübingen. The Department is one of the 7 sites of a multi-centre study, using electroencephalography (EEG) more amenable to clinical routine.

Methods: Following inclusion/exclusion criteria and informed consent, participants underwent series of auditory measures and on-line questionnaires (i.e., ESIT-SQ, several Visual Analog Scales, THI, TFI, HADS, PSQ-30, HQ) followed by EEG recording of the above-mentioned paradigm. We exposed participants to short sound pulses preceded or not by silent gaps embedded in a carrier sound of various frequency ranges (broad-band noise or BBN, 3 kHz and 8 kHz narrow-band noises). Trial Registration: NCT06520865.

Results: The paradigm was well tolerated by all participants. While nearly 75 % of suppression of pulse-evoked responses by gaps measured using MEG in a BBN carrier, we only achieved around 35 % using EEG, suggesting that EEG is less sensitive than MEG. Moreover, BBN were even less efficient than BBN in triggering inhibition. We present preliminary results from this first set of cases and controls and propose that BBN GPIAS may suffice as an objective outcome measure for tinnitus in humans using EEG, something that will be verified across the other clinical sites.

Conclusions: If successful, this novel approach may pave the way for an objective testing of drugs or medical devices with the aim of treating tinnitus.

M172. Upregulation of ATP-Purinergic P2rx2 Receptors in Hyperacusis in Cx26 Hetero-Deletion Mice and Attenuation by its Antagonists

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Background: As a common sensory disorder, hyperacusis can cause many hearing and psychological disorders, such as tinnitus, noise hypersensitivity, anxiety, learning disabilities, attention-deficit/hyperactivity disorder (ADHD), and other psychological abnormalities in the clinic. Our previous study demonstrated that Cx26 heterozygous deletion or mutation in the mouse and humans have hearing oversensitivity or hyperacusis (Liu et al., Sci Adv. 9, eadf4144, 2023, PMID: 36753545). However, little is known about underlying mechanisms. Also, there is no specific chemical treatment available in the clinic now. In this study, we found that ATP-purinergic P2rx2 receptors in the cochlea is upregulated after Cx26 deficiency and that administration of P2rx2 receptor antagonist can attenuate hyperacusis. This is the first time to reveal the role of ATP-purinergic signaling in hyperacusis generation, and also the first time to reveal an efficient chemical treatment.

Methods: Cx26 heterozygous deletion mice were used. Hearing function tests and hearing behavioral tests were recorded to assess hearing sensitivity and changes. RNA-Seq technique was employed to assess gene expression profile. Quantitative PCR (qPCR) and digital droplet PCR (dPCR) were used to confirm the gene expression changes. P2rx2 KO mice (Stock No 004603) were also used to test the effect of the downregulation of P2rx2 expression.

Results: Hearing function tests and hearing behavioral tests showed an enhanced hearing response or over-sensitivity in Cx26 hetero-deletion mice. RNA-Seq examination revealed a significant upregulation of ATP-purinergic P2rx2 receptors in the cochlea in Cx26 hetero-deletion mice. Such upregulation of P2rx2 was only found in the cochlea but not in the auditory cortex, inferior colliculus, and cochlear nucleus. There was also no upregulation of other P2rx receptors in Cx26 hetero-deletion mice. On the other hands, by crossing with P2rx2 KO mice to down-regulate P2rx2 expression, the hearing oversensitivity or hyperacusis in Cx26 hetero-deletion mice disappeared; the hearing function and behavioral tests became normal. Moreover, administration of P2rx2 receptor antagonist PPADS (pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid) by intraperitoneal injection eliminated hyperacusis in Cx26 hetero-deletion mice. Particularly, PPADS significantly reduced DPOAE, which was increased in Cx26 hetero-deletion mice. P2rx2 in the cochlea mainly expressed in the hair cells including outer hair cells (OHCs) and PPADS could affect OHC electromotility. These data supported the concept that P2rx2 may influence active cochlear amplification leading to hearing oversensitivity.

Conclusions: P2rx2 upregulation plays a critical role in the hyperacusis generation, which can be attenuated by administration of P2rx2 antagonists. This study is the first time to reveal the role of ATP-purinergic signaling in hyperacusis generation and also is the first time to reveal that P2rx2 antagonists can treat hyperacusis and possible other psychological disorders as well.

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M173. Generation of Hyperacusis by Overexpression of ATP-Purinergic P2rx2 Receptors in the Cochlea and Attenuation by Its Antagonists

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Background: Hyperacusis is a common hearing sensory disorder. However, little is known about underlying mechanisms. Our previous study found that Cx26 heterozygous deletion or mutation can produce hearing oversensitivity or hyperacusis (Liu et al., Sci Adv. 9, eadf4144, 2023, PMID: 36753545). Recently, we also found that ATP-purinergic P2rx2 receptors in the cochlea were upregulated and play a critical role in the hyperacusis generation in Cx26 hetero-deletion mice (see our co-submitted abstract). In this study, we used the AAV-based vector to overexpress P2rx2 in the cochlea and found that overexpression of P2rx2 in the cochlea can produce hyperacusis, which can also be eliminated by P2rx2 antagonist.

Methods: An AAV-based P2rx2 vector was injected into the right mouse cochlea via the posterior semicircular canal (PSCC) route. The expression of P2rx2 in the cochlea was examined by quantitative PCR (qPCR) and digital droplet PCR (dPCR) at the transcriptional level and by immunofluorescent staining with confocal microscopy at the protein level. Hearing function and hearing behavioral tests, such as ABR, DPOAE, CM (cochlear microphonics), and acoustic startle response (ASR), were recorded to assess hyperacusis generation.

Results: After overexpression of P2rx2 in cochlea by injection of AAV-based P2rx2 vectors, hearing function and behavioral tests showed hyperacusis generated in the injected mice. ABR, DPOAE, and CM recordings showed enhanced responses to sound stimulation. The oversensitive responses to sounds were only detected in the right injected ear but not in the left non-injected ear. Also, there was no hearing oversensitivity visible in the control mice with the same empty AAV vector (without P2rx2) injection. qPCR and dPCR showed that expression of P2rx2 in the right injected ears was 3-4 times higher than that in the left non-injected ears. Immunofluorescent staining also demonstrated that the staining for P2rx2 in the hair cells (including outer hair cells) was apparently intensive in comparison with that in the left non-injected ear or in the control mice with empty AAV-vector injection. As the same in the Cx26 hetero-deletion mice, administration of P2rx2 antagonist PPADS by intraperitoneal injection also reversibly eliminated hyperacoustic responses.

Conclusions: Overexpression of P2rx2 in the cochlea could generate hyperacusis, which could be eliminated by P2rx2 antagonist. This study further indicates that ATP-purinergic signaling has a critical role in the hyperacusis generation and possible other hearing and psychological disorders, such as tinnitus, noise hypersensitivity, learning disabilities, and other psychological abnormalities in the clinic.

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M174. Restoration of Hearing in Cx30 (GJB6) Knockout Mice by Compensation of Cx26 (GJB2) Expression in the Cochlea

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Background: Cx30 (GJB6) is another dominant gap junction connexin isoform and co-expressed with Cx26 (GJB2) in the cochlea. Cx30 mutations or knockout (KO) also could induce hearing loss. Previous studies demonstrated that Cx26 expression in the cochlea is reduced in Cx30 KO or mutation. In this study, we tested whether compensation of Cx26 expression in the cochlea could restore hearing function in Cx30 KO mice. This study provides valuable information for gene therapy for Cx30 mutation induced hearing loss.

Methods: Cx30 KO mice were used and injected with AAV-based GJB2 vectors via the posterior semicircular canal (PSCC) route. Only the right ear in the mouse was injected. Hearing function was assessed by ABR, DPOAE, cochlear microphonics (CM), and acoustic startle response (ASR) behavioral testing. RNA sequencing and digital droplet (dPCR) were also employed to assess gene expression and changes.

Results: After injection of AAV-based GJB2 vectors (right ear), the hearing function in Cx30 mice could be restored; the ABR thresholds in the right ear were reduced from ~100-110 dB SPL at the untreated Cx30 KO mice to ~30-40 dB SPL in GJB2-injected mice. DEOAE and CM in the injection ear were also recovered. The auditory behavioral rest measured by ASR recording also showed an almost normal response in the treated mice. However, the ABR thresholds in the left untreated ear remained at ~100-110 dB SPL; DPOAE and CM in the left ear were also not significantly improved. RNA-Seq and dPCR examinations showed that Cx26 expression in the cochlea was reduced after Cx30 KO and recovered to the normal level in the right ear after injection of Cx26 vectors.

Conclusions: Compensation of Cx26 expression in the cochlea can rescue hearing loss in Cx30 KO mice.

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M175. Valproic Acid Alleviates Blast-Induced Auditory Injury in Swine

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Background: Blast-induced hearing loss is a critical concern in the military settings, primarily due to the widespread use of explosive devices in combat. Despite advances in understanding blast physics and neurosensory pathology, effective pharmacological interventions remain limited. This study investigates the therapeutic potential of valproic acid (VPA), an FDA-approved drug, as a systemic treatment for blast-induced auditory injuries, with the goal of enhancing Services members' operational capabilities and quality of life.

Methods: Micro-Yucatan pigs (n=12, 12-14 kg, aged 2.5-4 months) were anesthetized with injection of Ketamine (20 mg/kg) and Dexmedetomidine (0.1 mg/kg) and were subjected to two blast exposures (BE) (peak static pressure of 23 psi with a 4 msec positive phase duration) in a Advanced Blast Simulator (ABS) in a prone position facing the oncoming shockwave, with a 2-minute interval between blasts. The blast overpressure was generated by Valmex membrane rupture in the ABS. Two hours post-blast, pigs received intravenous VPA. Auditory brainstem

responses (ABR) were measured pre-BE and at 1-, 7-, 14-, 21- and 28-days post-BE. Inner ears were histologically analyzed at 28 days post-BE. Cochlear histology and immunohistochemical analyses were performed at 28 days post-exposure to assess hair cell survival, synaptic integrity, and protein expression.

Results: Blast exposure caused a significant increase in ABR thresholds from a baseline of 35 ± 5 dB to 90 ± 10 dB at 1-day post-blast exposure ($p < 0.0001$), with sustained thresholds elevation (30–50 dB) and reduced ABR wave amplitudes from 4 kHz to 32 kHz frequency range through 28 days. VPA treatment significantly attenuated ABR threshold shifts, particularly in the high-frequency range (16–32 kHz, $p < 0.05$). Histological analysis revealed that blast induced a significant loss of inner and outer hair cells (IHCs and OHCs), particularly in the basal turn, with further reduced MYO7A protein levels and synaptic ribbon (CtBP2) number in surviving IHCs. VPA treatment significantly preserved OHCs in basal turn ($p < 0.01$), MYO7A+ IHCs in middle ($P < 0.05$) and basal turns ($p < 0.0001$), CtBP2 ribbons across all cochlear turns (apex: $p < 0.0001$, middle: $p < 0.001$, basal turns: $p < 0.0001$).

Conclusions: Blast exposure induces severe hearing loss and cochlear damages, including hair cell loss in the basal turn and the loss of synaptic connections. Systemic VPA administration post blast significantly mitigates these effects, preserving auditory function and cochlear integrity. These results highlight the potential of VPA as a promising therapeutic intervention for blast-induced auditory injuries.

M176. DFNA5: From Clinical Characteristics to Gene Editing Therapy Strategy

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Background: DFNA5 is a form of autosomal dominant, non-syndromic, progressive hearing loss. The clinical presentation, rate of hearing deterioration, and age of onset associated with DFNA5 mutations vary considerably. A thorough understanding of the characteristics and prevalence of DFNA5-related hearing loss is essential for the development of gene-based precision medicine targeting DFNA5.

Methods: We systematically reviewed the auditory characteristics of patients with DFNA5 pathogenic variants and investigated potential correlations between DFNA5-induced cell death and the onset of hearing loss. Additionally, we discuss the potential of inner ear organoids (IEOs) as a reliable alternative to current lack of a mouse models. We provide evidence-based recommendations for RNA-based gene editing strategies, highlighting their potential as therapeutic interventions with promising clinical applications. Data Sources. Ovid MEDLINE, Embase, and Web of Science

Results: To date, 17 distinct DFNA5 pathogenic variants have been reported, most resulting in exon 8 exclusion and the production of an aberrant exon 8-skipped protein with constitutive activity. Conventional DFNA5 knockout animal models do not reproduce human DFNA5 phenotypes including hearing loss, underscoring the need for alternative models to study the

disease pathology and develop new treatment. Recent advances in stem cell technology have enabled the generation of IEOs from human pluripotent stem cells harboring DFNA5 mutations via CRISPR/Cas9 gene editing. Targeting the exon 7–exon 9 junction using RNA-based systems shows promise in degrading aberrant exon 8-skipping transcripts, offering a potential approach to mitigate DFNA5-associated hearing loss.

Conclusions: This systematic review summarizes the clinical characteristics of DFNA5-related hearing loss and addresses current limitations in disease modeling. The proposed RNA-based gene editing strategy offers a novel therapeutic avenue for DFNA5-induced hearing loss and may provide insights applicable to other forms of genetic hearing loss (DFNAs).

M177. PUSH: Pipeline for Usher Syndrome Research

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Background: The Pipeline for Usher Syndrome Research (PUSH) at Boston Children's Hospital aims to tackle multiple forms of Usher Syndrome in parallel, identifying subtypes and mutations that may be amenable to innovative therapies. By advancing potential therapies, PUSH strives to identify optimized strategies that can move toward clinical trials, ultimately improving treatment options for hearing and vision loss associated with USH.

Methods: Patients with moderate to severe hearing loss were seen by pediatric otologists at Boston Children's Hospital. After confirmation of the clinical diagnosis, interested patients were enrolled for research through a broad-use consent and blood was collected. Exome sequencing or genome sequencing was performed, variants of interest were CLIA-confirmed, and Usher syndrome variant pathogenicity was established in accordance with criteria established by the American College of Medical Genetics. The team analyzed therapeutic targets for each patient and selected those most likely to be amenable to therapeutic intervention. Patient peripheral blood mononuclear cells were isolated from blood samples and iPSC lines were generated and used to produce inner ear and retinal organoids. Concomitantly, the team assessed different strategies to target the pathogenic USH mutations. Approaches included but were not limited to gene replacement strategies, splice correction and exon skipping with antisense oligonucleotides and gene editing. Initial work was performed on retinoblastoma cell lines, iPSCs, fibroblasts, or HEK cells expressing minigenes. Once a therapeutic approach was validated for a specific target, it was then tested in patient-derived inner ear and retinal organoids.

Results: We have identified 7 targets in three different USH genes. Three of seven targets have advanced through the pipeline with one validated on organoids.

Conclusions: PUSH provides a platform that will enable the rapid development of new therapies for Usher syndrome. The goal of the PUSH project is to accelerate therapeutic development from bench to bedside and streamline a scalable platform for development of individualized therapies for Usher syndrome.

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M178. Adult Expression of MYO15A Restores Growth of Stereocilia in Developmentally-Stunted Mutant Hair Bundles

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Background: Stereocilia are actin-based protrusions essential for hair cell mechanosensation. Mutations that impair their elongation during development cause profound deafness in both humans and mouse models. This includes mutations in Elongation Complex (EC) proteins, which drive stereocilia growth and establish row-specific hair bundle patterning. Early postnatal gene therapy targeting EC proteins WHRN and EPS8 showed promise, but adult AAV-mediated replacement failed to restore stereocilia structure, suggesting a narrow developmental window for effective intervention. Other EC components—MYO15A, GPSM2, and GNAI—cause similar phenotypes but their rescue has not yet been tested.

Methods: To assess rescue potential, we used the motor-deficient Myo15a shaker-2 allele. Because of the large Myo15a coding sequence, we engineered a Cre-inducible mouse model expressing the developmental isoform fused to HaloTag (ROSA26 DIO-HaloTag-Myo15a). Hair cell-specific Cre expression was driven either embryonically by Atoh1-Cre or induced in 6-week-old adults using tamoxifen-activated Gfl1-CreERT. Stereocilia were visualized by confocal microscopy, and their height and number quantified using Imaris software. Row organization was assessed by immunolabeling row-specific tip proteins. To supply the polarization module of the EC, we generated an AAV-PHP.B vector expressing HA-Gpsm2-P2A-Gnai3 and used posterior canal surgery.

Results: Early Atoh1-Cre-driven expression of HaloTag-Myo15a in shaker-2 mutants restored both elongation and row specificity, yielding apparently normal hair bundles. In Myo15a⁺ controls, Atoh1-Cre-driven overexpression caused exaggerated elongation following a tonotopic gradient, with shorter row 1 stereocilia at the cochlear base over elongating more dramatically than longer stereocilia at the apex. Adult induction of HaloTag-Myo15a did not alter normal bundles, but remarkably resumed elongation in stunted shaker-2 stereocilia. Two weeks post-induction, row 1 stereocilia reached normal height, though bundles lacked significantly graded heights and row-specific marker enrichment. EC proteins appeared de novo at all stereocilia tips and extra rows were modestly resorbed, suggesting partial reactivation of hair bundle differentiation. However, a 6-week chase period did not improve morphogenesis. Co-expression of HaloTag-Myo15a and AAV-HA-Gpsm2-P2A-Gnai3 failed to restore polarized GPSM2-GNAI enrichment at the hair cell surface, a prerequisite for restricting GPSM2-GNAI to row 1 and enabling asymmetric stereocilia patterning during development.

Conclusions: Late expression of Myo15a can drive elongation of stereocilia stubs that failed to develop, indicating that stereocilia growth is not temporally restricted. This growth can proceed independently of the GPSM2-GNAI-mediated planar polarization program, which normally establishes row-specific stereocilia heights and identities during development. These findings suggest potential for therapies aimed at regrowing or repairing stereocilia in adults. Increasing GPSM2-GNAI levels was insufficient to restore planar polarization within the hair bundle, explaining the near-uniform height and fate of shaker-2 stereocilia that elongate in adults. Auditory function was not rescued, probably due to the limited number of cells expressing HaloTag-Myo15a and extensive hair cell loss in the shaker-2 background.

M179. Preliminary Safety and Efficacy of AK-OTOF Gene Therapy for Otoferlin Gene (OTOF)-Mediated Hearing Loss

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Background: The otoferlin gene (OTOF) encodes otoferlin, a protein critical for signaling at the inner hair cell synapse. Individuals with mutations in OTOF typically present with congenital, severe to profound sensorineural hearing loss, with preserved otoacoustic emissions early in life. Recent advances in gene therapy and intracochlear delivery support the potential to restore physiologic hearing in individuals with OTOF-mediated hearing loss using a one-time, local administration of AK-OTOF (AAVanc80-hOTOF). The objective of this gene therapy trial for OTOF-mediated hearing loss is to assess the safety, tolerability, and bioactivity/preliminary efficacy of escalating doses of AK-OTOF.

Methods: Clinical trial participants have profound hearing loss at baseline as assessed by auditory brainstem response (ABR) and receive a one-time intracochlear administration of AK-OTOF using a minimally invasive transcanal approach and the Akouos Delivery Device. Safety assessments and auditory function tests, including ABR and behavioral audiometry, are performed over the trial and long-term follow-up periods.

Results: The preliminary safety profile is favorable, with no related serious adverse events and no adverse events related to AK-OTOF gene therapy or the Akouos Delivery Device. Initial clinical evidence shows that AK-OTOF gene therapy has the potential to restore physiologic hearing in individuals with OTOF-mediated hearing loss. Safety and efficacy data (through at least 3 months post-administration) from participants will be presented.

Conclusions: Available data suggest that AK-OTOF may be safely administered to participants, with onset of restoration of hearing as early as one month following administration.

M180. Audiological Characteristics Following Gene Therapy in Patients With Autosomal Recessive Deafness 9

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Background: Pathogenic mutations in the OTOF gene cause autosomal recessive hearing loss 9 (DFNB9), one of the most common forms of auditory neuropathy. Gene therapy shows promising potential for patients with autosomal recessive deafness 9 (DFNB9), with ongoing clinical trials (ChiCTR2200063181). A deeper understanding of changes in audiological characteristics is crucial for optimizing the monitoring and evaluation of patients' recovery post-treatment.

Methods: Audiological data were collected from 10 DFNB9 patients who underwent gene therapy, including auditory brainstem response (ABR), auditory steady-state response (ASSR), distortion product otoacoustic emission (DPOAE), and pure tone audiometry (PTA) tests.

Results: A clear ABR wave V was observed in all participants by 13 weeks. By 52 weeks, distinct ABR waves I and III were visible in some participants. The 1 kHz ABR wave V latency

at 85 dB decreased significantly from 9.220 [9.015, 9.810] ms at 4 weeks to 8.190 [7.780, 8.530] ms at 52 weeks ($p = 0.004$), with a trend toward increased wave V amplitude ($p = 0.055$). Significant correlations were observed between PTA, ABR, and ASSR thresholds at 0.5 to 4 kHz. The DPOAE signal-to-noise ratio (SNR) at 26 weeks post-treatment showed no significant difference compared with pre-treatment SNR values, nor were there significant correlations between the pre-treatment SNR values and the post-treatment ABR thresholds.

Conclusions: The study demonstrates that ABR and ASSR are reliable objective tools for assessing hearing recovery in DFNB9 patients after gene therapy. ABR reveals positive changes in the auditory pathway over time after gene therapy, enhancing our understanding of the impact of gene therapy on auditory pathway recovery.

M181. Comparative Analysis of RNA Versus Protein Splicing in Dual AAV-Mediated Gene Therapy in a Mouse Model of DFNB9 Deafness

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Background: Pathological mutations in the OTOF gene cause autosomal recessive deafness 9 (DFNB9). Although dual-AAV gene replacement therapy has been shown to partially rescue the hearing of patients with DFNB9, the therapeutic effects still need further exploration.

Methods: To investigate the impact of different recombination strategies on the efficacy of OTOF gene therapy, we constructed five dual-AAV1 therapeutic agents using RNA or protein splicing principles.

Results: Based on the recombination strategy using RNA splicing, the AAV1-AK system rescued the hearing of Otof^{-/-} mice to 55–70 dB, which outperformed the AAV1-AP and AAV1-TS systems. Based on protein splicing, the AAV1-intein system rescued the hearing of Otof^{-/-} mice to 35–70 dB, which outperformed that of AAV1-AK, and the effects lasted up to 12 months after injection in both newborn and adult mice. The efficacy of otoferlin re-expression and the number and functional restoration of ribbon synapses in the AAV1-intein system were also better than the AAV1-AK system.

Conclusions: These results show that protein recombination is more efficient than nucleic acid recombination for gene therapy in DFNB9. Therefore, this work not only provides data for optimization of DFNB9 gene therapy, but also provides a reference for the delivery of other large genes.

M182. Chemistry-Dependent Efficacy of Antisense Oligonucleotides in a Humanized DFNA9 Mouse Model

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Background: Deafness Autosomal Dominant 9 (DFNA9), caused by mutations in the COCH gene, is a dominantly-inherited adult-onset form of hearing loss. The common founder mutation c.151C > T (p.P51S) affects over 1500 individuals in Belgium and the Netherlands. Symptoms typically first appear between the 3rd and 5th decade of life, providing a window for therapeutic intervention. The non-haploinsufficiency disease mechanism suggests that blocking or reducing the p.P51S mutant cochlin protein levels may alleviate or prevent the DFNA9 phenotypes.

As such, we designed gapmer antisense oligonucleotides (ASOs) to specifically induce RNase H1-mediated degradation of COCH transcripts containing the c.151C > T mutation. ASOs are short, synthetic pieces of single-stranded RNA and/or DNA nucleotides that can specifically target RNA molecules. We employed different chemical modifications of the RNA wings to improve the affinity of these ASOs and their selectivity for the mutant transcript. Using overexpression in vitro models, we identified several gapmers that efficiently induce the degradation of mutant COCH transcripts.

The current study aims to evaluate these ASOs in vivo in our humanized DFNA9 mouse model to determine the most effective and safe candidate for further preclinical development.

Methods: Four candidate ASOs with different chemistries will be surgically delivered to the cochleae of humanized DFNA9 mice at a previously established safe and effective dose. Hearing function will be assessed by means of Auditory Brainstem Response (ABR) and Distortion Product Otoacoustic Emission (DPOAE) measurements. Mice will be treated unilaterally, with injected cochleae analyzed for mutant COCH transcript knockdown with qPCR. Contralateral cochleae, cerebrospinal fluid (CSF), kidneys and livers will be collected to assess potential ASO leakage with immunohistochemistry.

Results: Previous studies demonstrated that gapmers with 2'-OMe wings exhibited poor cochlear uptake and limited molecular efficacy, whereas gapmers with MOE wings performed significantly better. Previous studies also provided initial indications of ASO safety, as no increases in ABR or DPOAE thresholds were observed one week post-injection. Ongoing experiments are characterizing the intracochlear distribution of gapmer ASOs throughout the murine cochlea and performing a final in vivo comparison of the top-performing candidates to identify a lead molecule for further preclinical development.

Conclusions: Our in vitro evaluation of gapmer ASOs demonstrates that chemical modifications strongly influence allele-specific knockdown of mutant COCH transcripts. The ongoing final in vivo comparison of lead ASOs will identify a single, most effective and safe candidate for further preclinical development. These findings will provide a critical step toward a targeted, allele-specific therapy for DFNA9, with potential to prevent or alleviate progressive hearing loss in affected individuals.

M183. Development of New Video-Oculography Setup Paves the Way for Non-Invasive VOR Assessment in Mouse Models

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Background: The vestibulo-ocular reflex (VOR) stabilizes vision during head motion by generating compensatory eye movements opposite to the direction of head rotation. Impaired vestibular function disrupts this reflex. As such, quantitative VOR measurements provide an objective and reliable method to evaluate balance function in mice, offering clear advantages over subjective behavioral assessments. Traditional methods for assessing the VOR in mice require surgical attachment of a head cap to immobilize the animal, raising welfare concerns. In this study, we developed a non-invasive fixation system that securely holds the mouse during VOR testing without the need for surgery. This approach is expected to improve animal welfare and streamline VOR measurements for future studies.

Methods: Vestibular dysfunction was induced in CD-1 mice by allylnitrile administration, with untreated animals serving as controls. A non-invasive holder was developed to secure mice during video-oculography-based VOR measurements. VOR data obtained with this new setup were validated against behavioral assessments, including the Vestibular Deficit Index (VDI) and Forced Swimming Test. Measurements were performed at baseline (pre-allylnitrile), 1 week, 2 weeks and 8 weeks post allylnitrile administration. The reflex was measured during passive sinusoidal rotations of the head with frequencies of 1.67 Hz and 2 Hz, and rotational amplitudes of 10° and 20°. Hair cell pathology in the inner ear was further examined using immunohistochemistry.

Results: Initial VOR amplitude measurements were based on pupil tracking alone. To improve measurement accuracy, Pupil–Corneal Reflection (Pupil-CR) tracking was subsequently applied to correct for head and camera movement artifacts. VOR responses were then classified as present or absent and assessed at different timepoints across multiple stimulus conditions. At week 2, allylnitrile-treated mice exhibited fewer detectable VORs than controls across most conditions. By week 8, VOR responses were absent in all treated mice under most stimulus conditions, whereas control mice consistently showed detectable reflexes, particularly at higher amplitudes and frequencies, indicating persistent vestibular impairment due to allylnitrile treatment. These findings demonstrate that our VOR setup can accurately distinguish between control animals and those with vestibular deficits.

Conclusions: This study demonstrates that our newly developed, non-invasive VOR measurement setup provides a reliable and sensitive method for assessing vestibular function in mice. Allylnitrile-treated mice exhibited persistent and stimulus-dependent deficits in VOR responses, which were clearly distinguishable from control animals, confirming the system's ability to detect vestibular impairment. These findings highlight the potential of this approach to refine preclinical vestibular research and improve animal welfare by avoiding invasive procedures. Overall, this work establishes a validated tool for objective vestibular assessment and underscores its applicability for advancing preclinical vestibular research.

M184. Structural Neuroimaging of the Human Central Vestibular System: A Review

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Background: Vestibular disorders affect > 35% of adults over 40, yet the brain networks underlying vestibular processing remain incompletely understood. While functional neuroimaging reveals brain-wide activation during vestibular stimulation—with the temporo-parietal junction (TPJ) as a key cortical hub [1,2,3,4]—the underlying structural architecture supporting these networks remains poorly characterized. No systematic synthesis of structural neuroimaging has been done to define the anatomical underpinnings of the central vestibular network [5,6]. This review of 66 structural MRI (34 MRI, 32 diffusion-weighted MRI [DWI]) studies in humans addresses this major knowledge gap.

Methods: Google Scholar and PubMed were used to search for structural MRI studies. Inclusion criteria included human MRI and DWI studies from the year 2000-present on peripheral and central vestibular conditions: (1) bilateral vestibulopathy, (2) unilateral vestibular loss/deafferentation, (3) Meniere’s disease, (4) vestibular migraine, (5) post-traumatic/concussive vestibulopathy, (6) white matter disease/small-vessel lesions, (7) stroke/ischemic lesions in “vestibular circuits”, (8) chronic/mild vestibulopathy, (9) vestibular “experts”, (10) healthy adults (younger vs.older), (11) spaceflight-related vestibular adaptations, and (12) vestibular schwannoma resection. Each study was reviewed and summarized in two tables (one for MRI, one for DWI) with common criteria: Study Name, Vestibular Condition, Study Data, Outcome Measure, Association, and Brain Regions Involved.

Results: Despite heterogeneity across 12+ vestibular conditions, structural MRI findings converge on a brain-wide network, rather than isolated regions (Figure 1). The network overlaps with functional activation patterns, including the TPJ, and extends to additional regions supporting vestibular, motor, and cognitive integration. The 34 MRI studies showed: (1) gray matter changes were found in every major lobe across conditions, (2) the most frequently implicated regions were the hippocampus, cerebellum, insula, the parietal opercula, and regions within the TPJ. Paralleling the MRI-based findings, the 32 DWI studies reinforce network-level, rather than focal, changes: (1) microstructural/connectivity changes span a distributed network of regions, (2) frequently highlighted pathways include sensorimotor integration tracts (MLF, corticospinal tract, cerebellar peduncles), spatial cognitive networks (temporo–parietal–frontal circuits), and executive control circuits (frontoparietal and fronto-striatal tracts). However, heterogeneous findings likely reflect variety in vestibular conditions studied, small sample sizes ($n < 35$), methodological differences, and lack of longitudinal designs.

Conclusions: This systematic review of 66 structural neuroimaging studies is the first comprehensive characterization of the anatomical networks underlying vestibular processing. The convergent evidence for a distributed network spanning sensorimotor and cognitive circuits establishes a structural framework for understanding vestibular-brain-behavior interactions. Overall, these findings support developing structural neuroimaging biomarkers for tracking age-related changes, disease progression, and treatment response across the spectrum of vestibular conditions.

M185. Meta-Analysis of Structural Neuroimaging Studies in Vestibular Dysfunction

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Background: Vestibular dysfunction affects spatial orientation, balance, and memory, and has been linked to structural changes in cortical and subcortical regions of the brain. However, individual neuroimaging studies differ in sample size, modality, and region-of-interest (ROI) definitions, leading to inconsistent findings. A meta-analytical neuroimaging approach may be essential to quantify pooled effects and identify reliable neuroanatomical correlates of vestibular loss.

Methods: Following the meta-analytic approach of Manno et al. (2021), who reported gray matter alterations in hearing loss, we curated ROI-level effect sizes from 64 published structural MRI studies of vestibular dysfunction. Each entry represented a study $\times$ ROI $\times$ hemisphere contrast with standardized variance estimates. Reported effect sizes were converted to Hedge's g using small-sample corrections, allowing pooled estimation across studies. Our dataset spans 943 patients with vestibular dysfunction and 821 controls, across voxel-based morphometry, surface-based morphometry, and manual volumetry. Analyses focused on five ROI groups central to vestibular neurobiology: hippocampus, thalamus, basal ganglia, cerebellum, and temporal lobe. Random-effects models were used to compute pooled Hedge's g , 95% confidence intervals, and heterogeneity indices (Q , I^2). Cumulative meta-analysis was conducted by publication year, and meta-regression examined the influence of age.

Results: The hippocampus showed a significant pooled reduction (Hedge's $g = -0.65$, 95% CI $[-0.92, -0.38]$, $p < 0.001$; $I^2 = 48\%$), consistent with vestibular–hippocampal interactions supporting spatial memory. Some studies reported opposing effects across subfields (e.g., anterior reductions vs posterior expansions), highlighting the value of ROI-level forest plots in revealing heterogeneity. The thalamus showed consistent reductions across medial and pulvinar nuclei ($g = -0.42$, 95% CI $[-0.70, -0.14]$; $I^2 = 35\%$), underscoring its role as a vestibular relay. Cerebellar lobules VIII–X demonstrated robust atrophy ($g = -0.80$, 95% CI $[-1.10, -0.50]$, $p < 0.001$; $I^2 = 40\%$). Basal ganglia results were more heterogeneous ($g = +0.55$, 95% CI $[-0.05, 1.15]$; $I^2 = 62\%$), with both expansion and reduction across caudate and putamen. Temporal lobe results varied, with significant alterations in superior and middle gyri but less consistent effects across other subregions. Cumulative analysis revealed that early reports of hippocampal and cerebellar atrophy moderated in more recent, larger studies. Meta-regression suggested no significant relationship with participant age ($p > 0.2$).

Conclusions: This synthesis demonstrates converging evidence for structural alterations in hippocampus, thalamus, cerebellum, and temporal lobe in vestibular dysfunction, with more variable findings in the basal ganglia. The results emphasize the utility of ROI-level forest plots, which highlight heterogeneity and subregional dissociations often obscured by aggregated summaries.

M186. Epithelial–Mesenchymal Control of Otoconia Biogenesis and Macular Patterning in Human and Mouse Otolith Organs

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Background: Otoconia are biomineralized crystals in the saccule and utricle that provide the inertial mass for gravity and linear-acceleration sensing. Despite their essential role in balance—and their involvement in common disorders such as benign paroxysmal positional vertigo (BPPV) and age-related imbalance—the cellular programs for otoconia biogenesis and precise macular patterning remain obscure. Here, we investigate the role of the non-sensory “roof” domain of the saccule and utricle in coordinating otoconia production, routing, and macular patterning.

Methods: Calcified ~P30 mouse vestibular specimens were PMMA-embedded, sectioned 50–100 μm on a precision diamond wire saw, and imaged by brightfield and polarized-light microscopy to assess calcite birefringence. For evaluation of fine cellular and tissue morphology, mouse specimens were decalcified, celloidin-embedded, stained with PAS–alcian blue and imaged with confocal microscopy. Sections were deacrylated/de-celloidinized and immunolabeled for otoconial (OC90, DMP1) and ECM (fibronectin) proteins. One postmortem adult human saccule was osmicated, resin embedded, and imaged by FIB-SEM. 3D reconstruction was carried out to resolve matrix–cell interfaces. A previously published neonatal mouse utricle single-cell RNA-seq dataset was reanalyzed. Roof/transitional epithelium and adjacent mesenchyme were isolated and re-clustered for further downstream analysis, including differential gene expression, geneset enrichment analysis (ECM/secretory/biomineralization), and inferred epithelial–mesenchymal ligand–receptor crosstalk analysis. Key markers identified from RNA analysis were validated by immunohistochemistry.

Results: Two otoconia delivery modes were identified. In the saccule, an anterior roof epithelial domain (the human “reinforced area”) closely apposes bleb-shaped periepithelial mesenchyme. Both compartments exhibit strong OC90, DMP1, and osteopontin signals, and the mesenchyme contains calcium-rich vesicles. From this site, a fine fibronectin-rich ECM conduit—undetectable by standard cryo/paraffin methods—spans the endolymph to the anterior macula, carrying packets of immature crystals. FIB-SEM visualized secreted, matrix-like strands extending from the roof and nascent otoconia within or adjacent to the conduit. Disrupting this conduit in a saccular-hydrops model led to loss of macular otoconia, supporting a primary role in sourcing/organizing saccular crystals. In the utricle, roof epithelium broadly associates with melanin-containing cells; OC90, DMP1, and fibronectin delineate a diffuse, roof-derived protein “fog” that sediments onto the macula, where crystallization occurs on the surface.

Transcriptomic analysis identified three roof-associated cell states enriched for vesicular Ca^{2+} secretion, otoconial-matrix/biomineralization programs, and endolymph pH regulation. Ligand–receptor inference indicated robust epithelial–mesenchymal crosstalk with predominant mesenchymal-to-epithelial signaling, consistent with mesenchymal scaffolding of epithelial cargo.

Conclusions: These data support two conserved delivery modes—a directed ECM conduit in the saccule and a diffuse ECM “fog” in the utricle—coordinated by epithelial secretion and

periepithelial mesenchyme. These frameworks echo odontogenesis (tooth development), in which epithelial–mesenchymal interactions organize a mineralizing front. These findings highlight vulnerable processes that could contribute to BPPV and related balance disorders.

M187. Computational Modeling of Quantal Transmission Between Type II Hair Cells and Bouton Terminals

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Background: Amniote vestibular epithelia contain two main varieties of sensory cells that transduce head motion: type I hair cells and type II hair cells. Type I hair cells transmit to cup-like (calyx) terminals and type II hair cells transmit to compact (bouton) terminals of afferent neurons. Using a computational model we previously showed that changes in synaptic cleft potassium ion concentration ‘[K+]’ and electrical potential ‘ ϕ ’, drive post-synaptic currents during type I hair cell stimulation (Govindaraju et al. 2023) and underlie non-quantal transmission (NQT). However, most mammalian vestibular afferent neurons contact multiple type I and type II hair cells. Such “dimorphic” afferents with both calyx and bouton terminals receive both NQT and quantal transmission (QT) from their synaptic contacts. We do not yet know the functional significance of how quantal and non-quantal signals are integrated by these neurons.

Methods: To simulate type II cells, we incorporate data on major whole cell conductances (Martin et al. 2024) and mechanistically model QT. Our QT implementation simulates voltage-gated calcium channel (CaV) channel activation in hair cells, glutamate release into the synaptic cleft at discrete locations representing ribbon synapses, glutamate diffusion, and the kinetics of post-synaptic AMPA receptors as a function of glutamate concentration. We model quantal release as a stochastic process dependent on the magnitude of hair cell CaV channel current. Boundary conditions (ion concentrations and electrical potential) assume a perilymph bath where the afferent fiber exits the basement membrane of the epithelium (Govindaraju and Raphael 2025, bioRxiv preprint). Simulations were performed in COMSOL 6.3 software using a physiologically realistic finite-element geometry that permitted the incorporation of multiple type II hair cells and associated bouton fibers. The model predicts EPSCs and EPSPs in the afferent as functions of hair bundle deflection and can integrate signals from multiple bouton terminals contacting one or more hair cells.

Results: We present our implementation of QT in type II hair cells, and simulations of the afferent response for a varying number of bouton terminals contacting one or more type II hair cells. Parallel transmission to multiple boutons by an individual type II cell improves vesicle release probability, compensates for AMPAR desensitization, improves the magnitude and frequency of post-synaptic EPSCs. Likewise, the innervation of multiple type II cells by a single afferent also improves the chances of EPSC summation.

Conclusions: Our results support the hypothesis that having multiple bouton terminals of the same afferent contact a given type II hair cell can increase the frequency and magnitude of EPSCs. The architecture of dimorphic afferents and the innervation of multiple type I and type II hair cells appear to be series of adaptations to more quickly generate action potentials.

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M188. Signal, Noise, and Inference Among Vestibular Afferent Neurons

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Background: The foundation for understanding the dynamic response heterogeneity exhibited by vestibular afferent neurons results primarily from analyses focused on stimulus-evoked discharge modulation around the spontaneous discharge. Hence, the stimulus-dependent modulatory and stimulus-independent spontaneous discharge are the components of primary afferent spiketrains providing central vestibular circuits with information regarding head movements needed for gaze stabilization. A critical component of this processing scheme concerns the determination of afferent spiketrain components that confer head movement information from those that do not. The first steps in advancing the understanding of this process include determining the sources of noise in perstimulus afferent spiketrains, defined as components unrelated to the stimulus. An analytical framework to elucidate the contribution of noise in the spiketrain was previously advanced by considering measures of signal and noise in horizontal semicircular canal afferents. The objective of this investigation was to expand the generality of these analyses to afferents projecting from the superior cristae.

Methods: Spontaneous and stimulus evoked discharge were recorded from barbiturate-anesthetized chinchillas. Response spiketrains were recorded during yaw rotations of 0.8 and 1.6Hz with peak velocities representing isoacceleration stimuli. No adjustments to stimulus velocities were made to compensate for superior canal afferents out of the optimal stimulus plane. Signal and noise characteristics were derived from stimulus-response coherence, signal-to-noise ratio (SNR), and total harmonic distortion (THD). For these analyses afferents were categorized as either regular or irregular on the basis of their spontaneous discharge CV (regular: $CV < 0.1$; irregular: $CV \geq 0.1$).

Results: Superior canal afferents characterized as regular or irregular exhibited similarities in coherence, SNR, and THD, as previously found for horizontal canal afferents. However, because stimuli to superior canal afferents were out of the optimal stimulus plane their measured sensitivities were lower compared to horizontal canal afferents. Comparisons between superior and horizontal canal afferents were conducted separately for regular and irregular afferents using the Kullback-Liebler divergence as the test for similarities. Coherence and THD at 0.8 and 1.6 Hz were similar for regular afferents projecting from the superior and horizontal cristae, as were measures among irregular afferents. However, SNR was lower for both CV groups among superior canal afferents, consistent with the lower signal levels (reflected in response sensitivity).

Conclusions: These results further demonstrate the efficacy of the signal and noise analysis framework to elucidate the capabilities of vestibular afferents in transmitting high-fidelity estimates of head kinematics to the CNS. The inclusion of superior canal afferents to this framework extends the generality of the approach. This analytical framework is consistent with a unifying interpretation of sensory inference communicated by afferent signaling of head kinematic state that is readily processed by central Bayesian circuits. It is sufficiently robust to accommodate recently proposed alterations resulting from peripheral vestibular hypofunction.

M189. Meclizine, an H1 Antihistamine, Disrupts Roll-Axis Postural Control

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Background: Antihistamines are the largest class of pharmaceutical compounds used to treat and prevent vestibular disorders. Despite their efficacy, we do not know how their ability to suppress vestibular perception affects balance circuits.

Methods: Using a simple vertebrate model, the zebrafish, we show that a commonly-prescribed H1 antagonist, meclizine, modulates balance behavior.

Results: Strikingly, we observed that larval zebrafish swim upside-down during treatment with meclizine, implicating suppression of gravity-driven roll stabilizing postural reflexes. In contrast, H1 agonist exposure caused no change in posture, suggesting a ceiling effect on postural stability. Next, to understand changes to central vestibular processing after antihistamine administration, we imaged neural responses to body tilts in central vestibular circuits before, during, and after exposure to meclizine. While hindbrain neurons increased their response to roll-axis (leaning left and right) body tilts, preliminary data from downstream midbrain neurons showed changes in stimulus-evoked and spontaneous activity.

Conclusions: These results suggest that antihistamines act directly on central roll-stabilizing circuits. Future work using other drugs will reveal shared and/or divergent mechanisms of antihistamines. We anticipate that our model will generate new hypotheses for clinical studies with the long-term goal of shaping vestibular intervention strategies.

M190. Screening of Osteoporosis Drugs for Their Effects on Otolith Formation in Zebrafish

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Background: Benign paroxysmal positional vertigo (BPPV) is a common vestibular disorder believed to result from dislodged otoconia. Clinical studies suggest a relationship between BPPV, osteoporosis, and altered calcium metabolism; however, the mechanisms linking systemic calcium homeostasis to otolith formation remain unclear. Direct evaluation of otolith metabolism in humans is challenging because the inner ear is encased in the temporal bone and otoliths are minute structures. Zebrafish larvae provide a suitable model for otolith research because of their transparency, rapid development, and feasibility for pharmacological manipulation. This study investigated the effects of calcium-disrupting agents—cadmium and dexamethasone—on otolith formation, and evaluated whether osteoporosis-related drugs could modulate these changes.

Methods: Wild-type zebrafish (AB strain) embryos were collected and divided into treatment groups at 2 days post-fertilization (dpf). Larvae were exposed to cadmium (20, 40, 100 μ M), dexamethasone (20 μ M), or vehicle (DMSO). Additional groups were co-exposed to dexamethasone and osteoporosis-related agents, including ipriflavone and other clinically used anti-osteoporotic drugs, at three different concentrations. Larvae were fixed at 5 dpf and 8 dpf, bleached (1% KOH + 1.5% H₂O₂), and stained with Alizarin Red S. Otolith size (utricle and

sacculi) was measured under fluorescence microscopy. Whole-body calcium content was quantified by colorimetric assay after acid digestion of pooled larvae.

Results: Cadmium exposure significantly reduced whole-body calcium levels and resulted in smaller otoliths compared with controls. In contrast, dexamethasone decreased systemic calcium content but paradoxically induced otolith enlargement, accompanied by disruption of the layered internal structure of the otolith matrix. Screening of osteoporosis-related drugs revealed that most compounds did not significantly alter the dexamethasone-induced otolith hypertrophy. Notably, ipriflavone partially suppressed the enlargement of saccular otoliths, suggesting a potential protective effect on otolith calcification.

Conclusions: Cadmium and dexamethasone exert opposite effects on otolith morphology in zebrafish larvae, despite both reducing systemic calcium levels. These results highlight that local regulatory mechanisms, beyond systemic calcium balance, contribute to otolith morphogenesis. The partial protective effect of ipriflavone against dexamethasone-induced otolith hypertrophy raises the possibility that osteoporosis drugs may influence otolith homeostasis. Zebrafish larvae thus represent a useful experimental system for screening pharmacological agents that affect otolith formation, with implications for understanding the pathophysiology of vestibular disorders such as BPPV and their links to osteoporosis and drug therapy.

M191. Effects of Age and Carrier Frequency on AMcVEMPs

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Background: Despite advancements in vestibular assessment over the past three decades, our understanding of vestibular physiology remains incomplete. Novel methods can probe vestibular function in different ways and provide details of human vestibular physiology not yet reported. Amplitude-modulated cervical vestibular evoked myogenic potentials (AMcVEMPs) show sustained, phase-locked activity and non-linearity in sacculo-colic pathways. Assessing non-linearity in the vestibular system may have clinical implications, however several methodological factors require clarification before clinical application. Modulation frequencies that reliably elicit AMcVEMPs are known but the optimal carrier frequency remains uncertain, especially in aging populations.

This study examined age-related effects on AMcVEMPs elicited with bone-conduction at 250 Hz and 500 Hz carrier frequencies, focusing on amplitude, SNR, phase coherence, non-linear harmonic distortions, and response thresholds. Based on the reported frequency tuning of the otoliths in response to vibration, we hypothesized that 250 Hz would be a more effective carrier frequency.

Methods: Young (n = 31), middle-aged (n = 22), and older (n = 20) adults with no history of vestibular, neurological or middle-ear pathology participated. Stimuli consisted of 250 Hz and 500 Hz carrier frequencies, amplitude modulated at 37 Hz with a duration of 1024 ms, delivered via a B81 bone oscillator. Stimulus levels ranged from 65–40 dBHL for 500 Hz and 35–20 dBHL for 250 Hz, limited by B81 output at low frequencies. Participants turned their heads and maintained an ipsilateral sternocleidomastoid (SCM) contraction of ~50 μ V, while responses were recorded using surface electrodes. AMcVEMP responses were analyzed using Fast Fourier

Transform (FFT)-based methods. Non-linearity was assessed by examining responses at harmonics of the modulation frequency.

Results: Response thresholds were consistently lower at 250 Hz than 500 Hz carrier frequency: young adults (500 Hz: 52.9 dB HL; 250 Hz: 28.3), middle-aged (500 Hz: 61.2; 250 Hz: 31.4), older adults (500 Hz: 65.3; 250 Hz: 33.7). Response prevalence at 250 Hz was similar to 500 Hz despite lower presentation levels. For both carrier frequencies, aging was associated with significant reductions in amplitude, SNR, and phase coherence. Harmonic responses were present in most young, some middle-aged adults, and absent in older adults, indicating age-related loss of vestibular non-linear processing.

Conclusions: Carrier frequency of 250 Hz yielded lower thresholds with equivalent response prevalence, suggesting it is a more effective carrier frequency for AMcVEMPs. Overall, aging results in a significant decline in AMcVEMP responses across frequencies with reduced amplitudes as well as reduced non-linear harmonic distortion. Although B81 output is limited at low frequencies, higher-output transducers such as mini-shakers could enable characterization of responses at lower frequencies in broader, clinical populations.

M192. Integration of Vestibular Microstructures Into a Finite Element Human Head Model for Balance Research

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Background: The vestibular system plays a crucial role in human balance perception by detecting pressure gradients across the cupula in the semicircular canals and the otolithic layer in the utricle and saccule. These gradients drive mechanical responses that are translated into neural signals. Therefore, quantitative evaluation of pressure distributions within the vestibular system provides an essential means for understanding balance perception and its perturbations. However, previous finite element (FE) head models have primarily focused on auditory pathways, while detailed representation of vestibular microstructures has been limited.

Methods: In this study, we extended an existing three-dimensional FE human head model by incorporating anatomically informed structures of the cupula and otolithic layer. Their geometry and material properties were defined based on experimental data reported in the literature. The model was subjected to dynamic simulations under conditions equivalent to those described in prior inner-ear motion studies. To validate the model, simulated pressure gradients across the vestibular structures were compared with previously reported experimental and computational findings. Additionally, we examined the role of gravitational loading by introducing a static acceleration vector aligned with the gravity, thereby quantifying its impact on pressure gradient magnitude and orientation in the otolithic organs.

Results: The extended FE model reproduced the characteristic distribution and magnitude of pressure gradients across both the semicircular canal cupula and the otolithic layer. Quantitative comparisons showed strong agreement with earlier studies, confirming the reliability of the modeling approach. In particular, pressure differences across the cupula were consistent with values previously associated with angular acceleration, while gradients in the otolithic layer reflected sensitivity to linear acceleration. When gravitational loading was applied, the otolithic layer exhibited changes in both magnitude and directional alignment of the pressure gradient.

This result highlights the essential contribution of gravity to static vestibular stimulation and supports theoretical predictions of otolithic function.

Conclusions: By incorporating vestibular microstructures into a validated FE human head model, this study establishes a comprehensive framework for analyzing vestibular mechanics. The model provides experimental guidelines for future investigations into pressure gradient measurements within the vestibular system and offers new opportunities for integrated evaluation of auditory and vestibular stimulation. Such a tool is expected to support both fundamental vestibular research and the development of clinical or engineering applications targeting balance disorders.

M193. Social Determinants of Health Affect Clinical Outcomes Among Vestibular Rehabilitation Patients in South Florida

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Background: Vestibular rehabilitation therapy (VRT) is an exercise-based treatment that alleviates symptoms such as dizziness and imbalance for patients with vestibular disorders. However, adherence rates in the United States (US) remain under 50%. Disparities in access and outcomes thought to influence compliance and attrition remain poorly understood. While our previous work highlighted significant differences in VRT attendance across different insurance types (i.e., commercial, exchange/marketplace, Medicare/Medicaid, Military, Workers Comp), this study aims to examine how key social determinants of health may influence clinical response to therapy.

Methods: Through a retrospective chart review (IRB #20230698), we analyzed n=243 medical records for patients seen at the University of Miami Ear Institute who were prescribed VRT over a ten-year period (2012-2022). Patient-level data analyzed included insurance type, zip code, and clinical improvement in vestibular symptoms as measured by minimum clinical difference scores (MCDS) for the Dizziness Handicap Inventory (DHI) and Functional Gait Assessment (FGA), pre- and post-vestibular therapy. MCDS were ≥ 18 points for the DHI and ≥ 4 points for the FGA. SES was divided into low, middle, and high using median household income for each ZIP code, as reported by the US Census Bureau. Chi-square and ANCOVA tests were performed to examine the effects of sociodemographic factors on key outcomes.

Results: A total of n=243 patients were included. The majority were female (69%) and identified as White (81%) though nearly half (47%) were Hispanic/Latino. Seventy percent were 50 years or older at the time of referral. Overall, only 40% (n=98) of all patients participated in VRT, with an average of 8 (± 15) visits attended. Only 10% (n=10) of attendees had pre- and post-DHI scores and only 3% (n=3) had pre- and post-FGA scores recorded. Chi-square test of independence showed a significant association between SES and DHI response ($\chi^2=8.73$, $p=0.011$). Notably, none of the low-SES patients improved, compared with 64% of middle-SES and 50% of high-SES patients. Mean DHI change differed by SES, with low-SES showing minimal improvement (-2.5 ± 9.1), middle-SES substantial improvement (-20.0 ± 28.1), and high-SES moderate improvement (-18.5 ± 6.4). Following ANCOVA, we found that baseline DHI

scores were positively associated with outcomes ($F(1,22)=5.26$, $p=0.032$) when accounting for baseline differences in symptom severity. However, neither SES nor insurance category were significantly associated with positive VRT outcomes as measured by the DHI and FGA when accounting for presentation at baseline.

Conclusions: Socioeconomic status meaningfully shaped subjective outcomes after vestibular rehabilitation. Low-SES patients and several insurance groups showed no clinically significant improvement on DHI, while middle-SES patients demonstrated the greatest benefit. These disparities highlight that structural barriers, and not just clinical factors, limit recovery. Clinicians should account for social determinants in counseling and referrals, and system-level interventions are needed to ensure equitable access and optimize therapy effectiveness.

M194. Determinants of Referral Uptake and Outcomes in Patients Offered Vestibular Rehabilitation Therapy

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Background: Vestibular disorders are often managed with vestibular rehabilitation therapy (VRT), a specialized form of physical therapy utilized to address persistent visuo-vestibular symptoms that impact activities of daily living and quality of life. VRT is efficacious in treating peripheral vestibular disorders, as well as central pathologies like traumatic brain injury. Despite its efficacy, attendance and attrition rates vary widely, as patients face a multitude of challenges initiating or completing VRT. Understanding barriers to accessibility, including social determinants of health, is essential to ensuring equitable treatment and improving clinical outcomes for those affected.

Methods: A prospective survey (IRB#20230698) was conducted to investigate barriers to vestibular rehabilitation therapy (VRT) among adults with vestibular dysfunction. Participants previously referred for VRT between 2017-2025 were eligible if aged 18-77 with a diagnosis of traumatic brain injury, vestibular schwannoma, vestibular migraine, vestibular neuritis, or Meniere's disease. Eligible participants completed a Qualtrics survey by phone or email, covering clinical history, potential barriers to VRT, and socio-demographic data including age, ethnicity, marital status, education level, and annual income. Descriptive statistics were reported as mean±SD. Two-way ANOVA models were utilized to examine 1) symptom improvement (to test whether VRT participation was associated with greater clinical gains across predictors of interest and 2) symptom severity (to test whether baseline severity differed by VRT status and other predictors). Symptom scores and improvement were based on a self-reported Likert scale from 1-10. Post-hoc tests and estimated marginal means were applied where appropriate. Chi-square or Fisher's exact tests were used for categorical comparisons. Statistical significance was set at $p < 0.05$.

Results: Overall, we reviewed responses from $n=127$ individuals aged 55 ± 10 (range: 20-77) years of age, distributed among 105 (83%) females and 22 (17%) males. Participants primarily identified as White and non-Hispanic (88.9%). Vestibular schwannoma (39.5%), vestibular migraine (18.5%), and vestibular neuritis (17.7%) were the most common diagnoses, with 78.7% of subjects participating in VRT. Across both groups, the average symptom severity at baseline was 8.39 ± 1.94 and average symptom improvement was 3.65 ± 2.63 , regardless of participation.

Following ANOVA, we found that individuals who participated in VRT reported higher symptom severity at baseline ($p=0.03$). In age-stratified analyses, VRT participation was associated with greater symptom improvement in the 40-49 age group ($\beta=-2.86$, $SE=1.14$, $p=0.01$) as well as higher average symptom severity at baseline ($\beta=-1.88$, $SE=0.92$, $p=0.05$).

Conclusions: Overall, socio-demographics did not appear to affect self-perceived outcomes related to VRT. However, individuals who participated had greater and more timely resolution of symptoms, with age-specific advantages among a distribution of middle-aged participants. There appears to be a subjective baseline level of severity driving individuals to solicit VRT. Of note, our prospective survey was almost entirely female, which could relate to underlying psychosocial factors that affect clinical care.

M195. Effects of the Vestibulocochlear Implant on Balance and Gait in Bilateral Vestibulopathy

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Background: Bilateral vestibulopathy (BVP) often causes a range of debilitating symptoms. The resulting gait and balance problems significantly increase the risk of falls. The vestibulocochlear implant (VCI) is a neuroprosthesis that may offer a promising treatment for patients with severe BVP. This study aimed to explore the impact of different vestibular stimulation modes on gait and balance outcomes.

Methods: The VertiGo! trial is a prospective, nonrandomized, single-center cohort study. The trial includes nine patients with severe BVP who demonstrated inadequate compensation despite conventional treatment. Nine patients received the VCI and were extensively tested in four testing weeks, the first providing reference data (no VCI stimulation) followed by three weeks each with different stimulation modes: (A) baseline stimulation combined with head motion-modulated stimulation, (B) reduced baseline stimulation combined with head motion-modulated stimulation, and (C) only baseline stimulation, without modulation. For the gait and balance assessments, patients walked at 0.6m/s, 0.8m/s, and 1.0m/s on an instrumented treadmill integrated in a 6-DoF motion platform (CAREN; Motek). Five walking conditions were evaluated: unperturbed walking in both lit and dark environments, along with three sizes of pseudorandom mediolateral platform sway perturbations. Coefficients of variation (CoV) were analyzed using 3D motion capture data for step time, step length, double support time and step width. Patients also completed the Mini-BESTest each week.

Results: Step time, length and double support time CoV consistently reduced with increasing walking speeds across all walking conditions whereas step width CoV increased with increasing walking speeds across all walking conditions. Larger perturbations increased CoV consistently. The CoV in the reference mode and stimulation modes A, B and C varied across patients for all

gait parameters with no consistent effect for any one stimulation mode (e.g., 5 of 9 patients had lower step time CoV and step length CoV for one stimulation mode but these did not always align with the modes with lower CoVs in the other parameters and the stimulation mode that benefited one patient was often not the mode that benefited another patient). Darkness usually increased CoV but no clear effect of a specific stimulation mode in darkness was found. In the Mini-BESTest, at least one stimulation condition total score was higher than the reference total score for 6 of 9 patients but these were distributed across the different stimulation modes.

Conclusions: Walking speed, perturbations and stimulation modes can influence gait variability and balance performance. While specific stimulation modes may provide slight advantages during acute stimulation, individual responses exhibit considerable variability. The findings emphasize the importance of further investigating tailored stimulation. Investigation into potential differences between acute stimulation and ongoing stimulation is needed.

M196. Responses to Prosthetic Stimulation Remain Robust After Two Weeks of Continuous Ionic Direct Current Stimulation

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Background: The vestibular system in the inner ear detects head motion to maintain balance through reflexes like the vestibulo-ocular and vestibulo-colic reflexes (VOR and VCR). Previous work has shown the advantages of ionic direct current (iDC) over pulsatile stimulation due to its ability to excite, inhibit, and modulate vestibular pathways' sensitivity. Those acute experiments lasted up to 4 hours and used a separated interface nerve electrode (SINE) configuration. Without periodic replacement, that setup would be unsafe for chronic stimulation due to the production of chemical by-products that could alter pH levels at the tissue beyond safety limits. The effects of continuous iDC delivery to neural tissue have not yet been evaluated for safety. We developed a SINE device to continuously deliver iDC through replaceable gel-filled reservoirs via silicone tubing to fine microcapillary tips in the inner ear.

Methods: We injected both ears in adult chinchillas with the ototoxic drug gentamicin, then implanted microcapillary tips in the anterior semicircular canal of the inner ear's vestibular labyrinth. We used video-oculography (VOG) to measure electrically-evoked VOR (eeVOR) eye movements before and after animals received two weeks of 10-20 μ A continuous iDC using our head-mounted stimulator. The stimulator's electrolytic gel chambers were replaced daily for 2 weeks. During the two weeks of stimulation, VCR head tilt responses to 1-2 second step changes in current amplitude were measured hourly in free-roaming animals.

Results: Sensitivity to iDC stimulation varied across animals, as shown by both eeVOR and eeVCR measurements. Throughout the two weeks of constant iDC stimulation, animals consistently responded with head tilts to hourly changes in current amplitude. Head tilts were roughly aligned with the axis of the implanted semicircular canal and in phase with current changes. eeVOR responses after 2 weeks of stimulation varied but remained robust, with peak velocities exceeding 50 deg/s.

Conclusions: This study demonstrates that delivering ionic direct current to the inner ear over a prolonged period is possible without disrupting physiological function.

M197. First-In-Human Vestibular Implant Trial Update: Eye Movement Responses to Initial Device Activation

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Background: Bilateral vestibular hypofunction (BVH) is a disorder characterized by loss of balance and unstable vision that affects ~28/100,000 US adults. The Labyrinth Devices Multichannel Vestibular Implant (MVI) is a neurostimulator targeting the vestibular nerve branches innervating the three semicircular canals to restore balance sensation for those with BVH. Published results for the first 8 participants showed that the MVI improves measures of posture, gait and health-related quality of life.

Methods: From 2016-2025, 20 participants were implanted unilaterally with the MVI in a first-in-human study. In twelve participants, the device's single return electrode was implanted near the stimulating electrodes in the perilymphatic compartment of the labyrinth; the remaining eight have a distant return located outside the labyrinth. All underwent a device fitting procedure three weeks post-implantation which included reporting of perceptions of head motion and nontarget percepts (sound, facial twitch, or discomfort). Charge-balanced biphasic pulses (200 pps, 50-300 us/phase) were delivered, and currents were swept up to the threshold for eliciting nontarget percepts or the maximum device current of 700 μ A. Head-fixed electrically evoked vestibulo-ocular reflex eye movements were recorded, allowing selection of the best electrode and stimulation parameters for each canal.

Results: Eye movements $\geq 10^\circ/\text{s}$ about the targeted canal's axis were observed on the first day of stimulation for 53/60 implanted canals, and that number rose to 57/60 at return visits. The group median [95% confidence interval] maximum eye velocity was $53.9^\circ/\text{s}$ [39.1-77.7] with misalignment from the target axis of 23.3° [19.1-26.5]. Stimulus current was constrained below the device's maximum of 700 μ A at 100 $\mu\text{s}/\text{phase}$ by nontarget percepts in 8/106 (8%) of electrodes with a near return and 41/72 (57%) with a distant return; however, using a distant return did not negatively impact evoked eye movements. Eye velocities and misalignment were similar for those with a near return ($51.5^\circ/\text{s}$ [36.4-95.4], 23.3° [16.7-26.5]), distant return ($54.3^\circ/\text{s}$ [23.5-90.8], 22.8° [15.4-37.8]), ototoxic BVH ($54.6^\circ/\text{s}$ [39.1-95.4], 23.3° [14.5-26.8]), and non-ototoxic BVH ($52.0^\circ/\text{s}$ [25.8-90.8], 23.0° [17.3-30.5]).

Conclusions: Vestibular stimulation elicited canal-aligned eye movements in almost all implanted canals that were similar with a near or distant return, with the former generally requiring higher currents and eliciting fewer nontarget percepts. Responses were also similar for participants with both ototoxic and non-ototoxic BVH. These results indicate that MVI stimulation is effective in eliciting VOR responses in patients with BVH with a variety of etiologies.

M198. Effect of Head Movement on Cybersickness: A Pilot Study Using a Wearable Motion Sensor

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Background: Cybersickness is a critical issue associated with the increasing use of virtual reality (VR). It is thought to result from sensory conflicts between visual input and vestibular or somatosensory signals. Whether head movement during VR exposure mitigates cybersickness remains unclear.

Methods: Healthy adults were randomly assigned to either a head-fixed group or a head-movement group. Participants viewed a 10-min drone-recorded VR video using a head-mounted display. Head kinematics were monitored with a Foster RN002 TW wearable triaxial accelerometer. Postural stability (stabilometry before and after VR), and Simulator Sickness Questionnaire (SSQ) were assessed.

Results: The head-fixed group showed greater postural sway after VR exposure and higher SSQ scores compared to the head-movement group. In contrast, the head-movement group maintained postural stability and reported lower SSQ scores. Wearable sensor data clearly distinguished between the two groups.

Conclusions: Allowing head movement during VR may reduce cybersickness, and wearable ear-level sensors provide objective quantification of head motion. This pilot study suggests a potential strategy for mitigating cybersickness and for developing novel assessment tools.

M199. Decreased Response and Tuning Shift of the cVEMP Suggest the Propagation of Excessive CSF Accumulation on Saccules in Idiopathic Normal Pressure Hydrocephalus

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Background: This study aimed to investigate the effects of excessive cerebrospinal fluid (CSF) retention on peripheral vestibular function and inner ear fluid condition in patients with idiopathic normal pressure hydrocephalus (iNPH). iNPH is characterized by a clinical triad of gait and balance disturbances, cognitive impairment, and urinary incontinence in older patients. CSF pressure in patients with iNPH is normal, but intermittently increases slightly. Although gait disturbance is the most common symptom of the triad, its etiology remains unclear, and only a few reports have reported vestibular examination findings in iNPH.

Methods: Cervical vestibular evoked myogenic potential (cVEMP) was measured in 25 patients with iNPH before the spinal tap test (10 males and 15 females, mean age [SD]: 77.0 [6.0]). The p13-n23 amplitude and asymmetry ratios (ARs) in 500 Hz short-tone burst stimuli were evaluated as part of peripheral vestibular function. The tuning properties of 500 Hz and 1,000 Hz short-tone burst stimuli of cVEMP were evaluated as factors that may reflect the condition of the

inner ear membrane labyrinth. Furthermore, cVEMP was measured in 12 age-matched non-iNPH patients as controls.

Results: Only five of the 25 patients with iNPH experienced vertigo or dizziness in the past. Ten of the 25 (40%) patients with iNPH exhibited vestibular dysfunction in the cVEMP by bilateral absent response or increased ARs ($> 24.89\%$, institutional standards). cVEMP tuning was significantly shifted to a higher frequency in patients with iNPH than in age-matched controls (mean [SD]: 1.84 [0.92] and 1.03 [0.27] in the iNPH and control groups, respectively; $p=0.0047$, Mann-Whitney U test). Furthermore, the cVEMP tuning shift improved in seven of nine patients who were reassessed after shunt implantation.

Conclusions: The improvement in cVEMP tuning shift after shunt implantation suggests that excessive CSF accumulation in iNPH may extend to the endolymph or perilymph surrounding the saccules. Saccular dysfunction may be a possible cause of iNPH imbalance.

M200. Two-Photon Imaging of Tilt-Evoked Vestibular Cortical Activity in Mice

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Background: For over a century, researchers have developed specialized devices—from early observational chambers to modern six-degree-of-freedom platforms and wireless systems—to record brain activity in response to movement. Each setup involves trade-offs in cost, accessibility, and flexibility. Only a few recent electrophysiological methods support repeated, reliable measurements from neuronal populations. Although genetically encoded calcium indicators enable such recordings, their use in vestibular research has been limited by technical challenges. Recent advances like rotating microscopes and imaging rotating samples show promise, but require specialized expertise. Here, we adapted a method originally developed for imaging brainstem responses to tilt in zebrafish (Hamling et al., 2023) for use in mice. Using two-photon imaging, we show that this low-cost, simple approach allows reliable recording of vestibular cortical responses in mice.

Methods: We used an adeno-associated virus (AAV) to express the red fluorescent, genetically encoded calcium indicator R-GECO in neurons of the retrosplenial (RSC) and posterior parietal (PPC) cortices of C57BL/6 mice. These areas are easily accessible and are known to receive strong vestibular inputs (Hennestad et al., 2021). We performed simultaneous two-photon imaging of both hemispheres using a Nikon system through a transparent cranial window (Deemyad et al., 2025). Mice were head-restrained in a rotatable tube. A one-minute baseline recording was followed by a 20° nose-down tilt for 15 seconds (stimulus period, no imaging). The animal was then rapidly (< 1 s) returned to the initial horizontal position, and the same neurons were imaged for one minute (response period). An additional one-minute recording confirmed a return to baseline activity (post-stimulus period). This protocol was repeated five times per session. Active neurons were identified during the response period and tracked across all periods using the AQuA2 platform (Mi et al., 2025).

Results: Our preliminary results show that neurons in both vestibular cortices respond with reliable increases in activity across multiple trials. We analyzed neurons that responded to at least 4 out of 5 trials. In the response period, there was an initial increase in neural activity,

reaching a peak value of $0.30 \pm 0.03 \Delta F/F$ ($n = 7$, averaged over 5 trials). This was followed by an exponential decay with a time constant of 1.8 ± 0.4 s and returned to 'baseline' values ($0.019 \pm 0.008 \Delta F/F$) in approximately 3 s.

Conclusions: Adapting a method from zebrafish (Hamling et al., 2023), we show that activity in vestibular cortical neurons can be reliably recorded in mice after a tilt. This approach could potentially provide chronic recordings from the same neurons, enabling robust studies of vestibular processing in a mammalian model.

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M201. Performance on the Gait Disorientation Test in Fallers and Non-Fallers With Hearing Loss

Erin Hernon^{*1}, Erin Piker², Brandy Hollins², Hannah Martin¹, Nathan Sattah¹, Courtney Hall³, Kristal Riska¹

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Background: The gait disorientation test (GDT) measures the difference in time to walk with eyes open versus eyes closed, where a greater difference indicates more difficulty navigating without vision. Recent studies of the GDT have demonstrated moderate to strong associations with measures of balance ability, balance confidence, and dizziness handicap. As a diagnostic screener, it has excellent discriminative ability to identify patients with peripheral vestibular hypofunction. To our knowledge, performance on the GDT has not yet been evaluated as a measure to identify fall risk. The purpose of this study was to investigate if there was a significant difference in gait disorientation score between fallers and non-fallers in older adults with no known vestibular impairment.

Methods: Adults aged 60 years and older with hearing loss and no known history of vestibular disease who were able to walk 10 meters unassisted were recruited for the study. Participants completed the Functional Gait Assessment (FGA), which consists of 10 walking balance tasks. Items 1 and 8 require the participant to walk 6.096 meters with their eyes open and closed, respectively. The gait disorientation score was calculated by subtracting the time in seconds to complete FGA item 1 from the time to complete FGA item 8. The cohort was divided into those who had fallen in the 12 months prior to testing (i.e., "fallers") and those who had not (i.e., "non-fallers"). A Mann-Whitney U test was used to compare gait disorientation scores between groups.

Results: 179 participants were enrolled in the study. However, 1 participant was unable to complete FGA item 8 due to imbalance and was therefore excluded from analysis. The faller group ($n = 90$) was predominantly white (84.4%), female (65.6%), and had a mean age of 72.24 ± 6.64 years. The non-faller group ($n = 88$) was predominantly white (81.8%), female (51.1%), and had a mean age of 70.70 ± 5.84 years.

The mean gait disorientation score was 2.66 ± 3.50 seconds in the faller group and 1.13 ± 1.50 seconds in the non-faller group. A Mann-Whitney U test indicated significantly greater gait disorientation scores in the faller group compared to the non-faller group, $z = -2.998$, $p = .003$.

Conclusions: Fallers had significantly greater gait disorientation scores compared to non-fallers, indicating that fallers have larger gait discrepancies between walking with their eyes open versus closed. This suggests that even when vestibular function is normal, fallers may weigh visual cues more heavily than non-fallers during spatial navigation. Further research is needed to understand the diagnostic ability of the measure to predict fall risk in older adults.

Key takeaway: Compared to non-fallers, fallers experience greater changes in gait speed when walking with vision versus without vision.

M202. Quantifying the Vestibulo-Ocular Reflex (VOR) in Aro Chick Model of Congenital Vestibular Disorders

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Background: Syndromic congenital vestibular disorders (CVDs) feature a sac-like inner ear with truncated or absent semicircular canals, yielding deficits in balance and gaze stabilization in CVD patients. To study CVDs, our group implemented a chick model that forms a sac-like inner ear as seen in CVD children by 180° Anterior-posterior Rotation of the Otocyst surgically on one side in 2-day-old chick embryos in ovo, the ARO chick. As the vestibular pathways are largely conserved among vertebrates, the ARO chick model offers an opportunity to study the pathophysiology of CVDs.

Methods: VOR performance for gaze stabilization was measured in ARO hatchlings and compared to age-matched normal hatchlings (H) from 5-10 days after birth (H5-H10). Hatchlings were placed on a motorized turntable (Newport Corp.) with the head fixed in a natural position. Horizontal eye movements were recorded using an infrared camera (ISCAN) during table rotations performed at velocity < 30°/s and frequencies < 1Hz. Sinusoidal VOR traces were divided into half cycles to differentiate rotation toward the operated and unoperated ear. Table rotations were limited to 30-60 seconds during sessions repeated in the morning and afternoon, alternating right and left eye recordings. To avoid animal fatigue and discomfort, sessions were restricted to < 15 min. Our preliminary data reported here is based on experiments performed on over 10 hatchlings to develop the protocol and obtain consistent VOR recordings.

Results: Like normal chicks, ARO chicks can stand shortly after birth, but show head-tilt toward the rotated side. We successfully implemented eye movement recordings of the hatchling chicks using for the first time non-invasive video-oculography imaging. In dim light, VOR responses evoked by sinusoidal rotations in the yaw plane were composed of typical compensatory slow phases interrupted by rapid quick phases. Normal chicks presented a VOR gain in the range of 0.3–0.6, which increased with frequency, and a phase response in the range +/-10° at 0.2–1 Hz. Compared to normal chicks, VOR gains in ARO chicks were reduced at higher frequency by about 1/3 (0.36 at 1 Hz) for both operated and non-operated sides. Phase responses were in the normal range, indicating relatively preserved timing of the compensatory eye movements.

Conclusions: In newly-hatched ARO chicks, compensatory eye movements appear qualitatively preserved compared to normal chicks. However, decreased VOR gain in ARO chicks suggests gaze instability during head movements when stimulation reaches the higher dynamic range. Ongoing studies will further test the higher dynamic range with frequencies up to 2 Hz and velocity up to 100°/s where additional deficits may be apparent. We will compare ARO chicks

with simple sacs to those forming truncated canals and measure the otolith-dependent reflex by performing tilt/roll stimulations.

M203. Solving the Structure of TECTB: Purification and Analysis of a Hearing Loss-Associated Variant

April Keyes^{*1}, Neil Sash², Pedro De-La-Torre³, Evan Hale⁴, Artur Indzhukulian⁵

¹*Massachusetts Eye and Ear*, ²*Massachusetts Eye and Ear, Eaton Peabody Laboratories*, ³*Harvard University & Massachusetts Eye and Ear Infirmary*, ⁴*Speech and Hearing Bioscience and Technology Program, Harvard University*, ⁵*Harvard Medical School, Massachusetts Eye and Ear*

Background: Extracellular matrix that overlays the sensory hair cells of the inner ear. β -Tectorin (TECTB) is a major non-collagenous component of the TM and contributes to the formation of the striated-sheet matrix (SSM) through interactions with several TM proteins, many of which are established deafness genes. Despite its importance, the atomic structure of TECTB remains unknown. Recently, we reported a missense variant in TECTB (C225Y) segregating with autosomal-dominant hearing loss in a multigenerational Nicaraguan family. In a knock-in mouse model carrying TECTB-C225Y, we showed profoundly disruption of SSM assembly despite the presence of TECTB protein within the TM, leading to impaired cochlear resilience and hearing dysfunction. This finding highlights the need for structural insight into TECTB to understand how disease-associated variants may disrupt SSM assembly and TM function.

Methods: To enable structural studies, we are developing a pipeline to express and purify recombinant wild-type and TECTB-C225Y protein. cDNA constructs encoding N-terminally-tagged TECTB are transformed into a bacterial expression system, induced, and harvested for protein isolation. Cell pellets are lysed, and lysates are subjected to Ni-affinity and size-exclusion chromatography to enrich tagged TECTB and remove aggregates and contaminants. Purified fractions are then evaluated by SDS-PAGE, concentrated, and screened across a range of buffer conditions to identify formulations suitable for crystallization.

Results: Preliminary experiments demonstrate successful recombinant expression of wild-type TECTB and recovery of protein following lysis. Ni-affinity chromatography has produced fractions with a prominent band at the expected molecular weight of TECTB, with a limited number of additional bands detected by SDS-PAGE. The initial size-exclusion chromatography indicates that a subset of the protein elutes in a monomeric state, alongside earlier-eluting species consistent with oligomeric or aggregates. Ongoing optimization focuses on buffer composition and purification parameters. In parallel, cloning and expression of the TECTB-C225Y variant are underway to enable direct comparison with wild-type TECTB.

Conclusions: These preliminary data establish the feasibility of recombinant expression and purification of TECTB for structural studies. Given the demonstrated failure of SSM assembly in Tectb-C225Y mutant mice, defining the structure of TECTB—and how the C225Y substitution alters it—will be critical for understanding the molecular basis of SSM formation and its disruption in hearing loss. Ultimately, comparative structural analysis of wild-type and mutant TECTB is expected to provide mechanistic insight into how TM matrix assembly fails in disease.

Mini Podium 1: Psychoacoustics in the Service of Active Auditory Perception

3:00 p.m. - 4:00 p.m.

Meeting Room 209ABC, Floor 2

Moderators: Petra Kovacs and Andrew Oxenham

Active Audition Improves Perceptual Accuracy and Metacognitive Sensitivity

3:00 p.m. - 3:15 p.m.

Ziying Tang¹, Dardo Ferreiro¹, Valerie Herbert¹, James Dowsett², Paul Taylor³, Michael Pecka*¹

¹*LMU Munich*, ²*University of Sterling*, ³*University of Zurich*

Background: In everyday life, whether following a conversation in a bustling café or localizing a car honk on a busy street, we never simply passively absorb sounds. Instead, real world hearing can be best described as “active audition”: a multi-stage process in which listeners integrate top-down predictions with bottom-up sensory evidence to form a perceptual judgment. A metacognitive evaluation determines confidence in that judgment, which in turn guides whether the listener commits to a decision or actively seeks additional sensory evidence through goal-directed actions. Thus, metacognition - our capacity to monitor and evaluate the reliability of our own perception - is central for appropriate action selection to enhance sensory information and ultimately facilitate accurate perception. However, active audition is typically not reflected in laboratory/clinical hearing tests. Here, we systematically investigated the influence of active audition on frequency discrimination and metacognition using the Sensory Island Task for humans (SITh) together with mobile EEG recordings.

Methods: Twenty normal-hearing adult participants engaged in three experimental conditions: active exploration via locomotion (walking in an arena), active searching with a computer cursor, and passive listening. In each trial, participants were tasked with identifying a target frequency among distractors and providing confidence judgments, allowing for analyses of both perceptual accuracy and metacognitive sensitivity (AUROC2).

Results: Results show that both active conditions led to significantly higher perceptual accuracy and reduced false alarm rates compared to passive listening, confirming enhanced performance through active audition. Preliminary EEG analyses revealed that signal-to-noise ratio of Steady-State-Auditory-Evoked-Potentials were higher under active audition compared to passive listening. Moreover, locomotion elicited significantly greater peak-to-peak amplitudes than stationary or passive listening. Interestingly, mean confidence ratings did not differ between conditions, and overall metacognitive sensitivity showed pronounced individual differences but no systematic enhancement through active sensing. Passive listening was associated with greater variance in AUROC2, especially among male participants, indicating more heterogeneity in the relationship between confidence and accuracy when exploration was absent.

A central finding was that musical ability correlated strongly with metacognitive precision specifically in the active conditions. Participants who performed better on the Musical Ear Test (MET) not only achieved higher accuracy during active conditions, but also demonstrated superior metacognitive sensitivity. This relationship was absent in the passive listening condition, indicating that musical competence selectively supports metacognitive precision when participants engage actively with the auditory environment.

Conclusions: These findings endorse the role of closed-loop sensory-motor feedback in boosting both sensory efficiency and evaluative processes during active frequency discrimination, while highlighting that musical ability acts as a moderator for metacognitive precision primarily under conditions of active engagement.

Optimized Models of Uncertainty Explain Human Confidence in Auditory Perception

3:15 p.m. - 3:30 p.m.

Lakshmi Narasimhan Govindarajan^{*1}, Sagarika Alavilli², Josh McDermott¹

¹*Massachusetts Institute of Technology*, ²*Harvard University*

Background: Sensory inferences about the world are made from ambiguous observations and are thus inevitably uncertain. Accurate estimation of uncertainty is likely critical to decisions about where and when to act, but little is known about how uncertainty is estimated for real-world perceptual problems. To investigate this issue, we developed a new class of stimulus-computable models that represent uncertainty of estimated perceptual variables. We optimized these models for sound localization and pitch estimation, and compared their uncertainty estimates to human confidence judgments obtained from experiments in which participants reported confidence on a trial-by-trial basis.

Methods: The model estimated parameters of a probability distribution over the variable to be estimated (location or fundamental frequency for the present work). The models were optimized to maximize the likelihood of ground truth labels for large datasets of natural sounds, utilizing binaural spatial renderings for localization and short excerpts of speech or music for pitch estimation. When stimuli are ambiguous, the models should learn to produce wider or potentially even multi-modal distributions. To measure human confidence, we asked participants to make localization or pitch judgments and place monetary bets (1–5 cents) to indicate confidence. To simulate the same experiments on the models, we took the model’s confidence to be the entropy of its posterior distribution (mapped onto a scale of 1-5 cents via a monotonic, deterministic function).

Results: Human confidence judgments varied with stimulus properties: lower bets were placed for peripheral locations and pure tones in localization, and for complex tones with higher-numbered harmonics in pitch. These variations in confidence across conditions were largely mirrored by the model in both domains.

Conclusions: Humans have internal estimates of the uncertainty of their auditory percepts—both for sound location and pitch. These confidence judgments closely match the uncertainty estimates of models optimized for accurate performance in each domain, suggesting that human

confidence is normatively appropriate. This modeling framework offers a general approach for investigating confidence across diverse perceptual domains.

Disentangling the Mechanisms of Behavioral Noise Adaptation

3:30 p.m. - 3:45 p.m.

Enrique Lopez-Poveda^{*1}, Andrea Viguera-Losada¹, Milagros J. Fumero¹, Miriam I. Marrufo-Pérez¹, Almudena Eustaquio-Martín¹

¹*University of Salamanca*

Background: Noise adaptation refers to the improvement in hearing function as the sound of interest is delayed in the noise. The mechanisms underlying noise adaptation are still unclear, but previous studies have proposed that adaptation could result from noise activation of olivocochlear (OC) reflexes, neural dynamic range adaptation (DRA) to the noise level (reviewed by Marrufo-Pérez and Lopez-Poveda 2022, JASA 151:1741-1753), and/or the formation of noise schemas (Sch) (Hicks and McDermott PNAS 121:e2408995121). Here, we present an approach and preliminary data aimed to disentangle the importance of these mechanisms for noise adaptation.

Methods: Our approach assumes that the three mechanisms may aid hearing in noise and may interact with one another, depending on the type of noise. 17 women with normal hearing were asked to recognize 11 vowel-consonant-vowel (VCV) tokens embedded in 39 different noises. Each word was presented five times in each noise. The word and the noise varied from trial to trial. Measurements were made in the presence and in the absence of 1-second noise precursors. Adaptation was quantified as the improvement in recognition caused by the precursor. Crucially, adaptation was compared for precursors designed to gradually activate the mechanisms of interest. To measure the effect of OC reflexes alone, precursors fluctuated in level every 50 ms to prevent them from causing DRA and differed from the simultaneous noise to minimize noise schemas from facilitating recognition. To measure the joint effect of OC reflexes and DRA, precursors were like OC precursors but were steady in level. To measure the joint effect of the three mechanisms on adaptation, precursors were like OC+DRA precursors, and the simultaneous noise was of the same type as the precursor noise. Word and noise levels were 45 dB SPL and 60 dB SPL, respectively, i.e., the signal-to-noise ratio (SNR) was -15 dB. The mean level of the precursors was set equal to the noise level.

Results: Mean adaptation was gradually greater for the OC precursors (0.4%), the OC+DRA precursors (1.5%), and the OC+DRA+Sch precursors (2.5%). However, statistically significant adaptation occurred only for the OC+DRA+Sch precursors. Adaptation was small probably because the measurements were made at a fixed SNR and speech reception thresholds varied across individuals.

Conclusions: These preliminary results suggest that the three mechanisms may contribute to behavioral adaptation to noise. Further research is necessary to refine the methods and confirm these findings. (Work supported by the Spanish MICIU, ref. PID2024-158683NB-I00).

An Association Between Vocal Perception and Vocal Production in Marmoset Phee Calls

3:45 p.m. - 4:00 p.m.

Michael Osmanski*¹, Hrishikesh Mandal¹, Xiaoqin Wang¹

¹*Johns Hopkins University*

Background: How animals segregate environmental objects into discrete, behaviorally meaningful categories, and how animals rely on those categories to choose relevant environmental stimuli to respond to, is one of the hallmark questions in behavioral biology. Previous work in our lab has shown that marmosets appear to have perceptual specializations for grouping their species-specific calls into natural categories. Specifically, marmosets show reduced sensitivity to changes in the acoustic features of their calls when those features fall within the natural range of variability of those calls (based on vocal recordings from our large colony) compared to when those features fall outside of the natural range of variability. This is a phenomenon also seen in human speech perception, and suggests that marmosets ignore variability in call parameters if that variability falls within a call category boundary. Here, we ask whether these natural perceptual categories are also reflected behaviorally through vocal responses recorded during antiphonal calling interactions. Marmosets routinely engage in antiphonal calling, a cooperative vocalization pattern between visually occluded conspecifics in which individuals reciprocally exchange phee calls.

Methods: In this experiment, we utilized previously recorded natural phee calls and also synthetic phee calls with dominant frequencies that either fell within or outside of the natural range of phee calls to determine the relationship between perceptual categories and antiphonal responsiveness. We used a computer-controlled interactive playback paradigm which engaged marmosets in antiphonal calling bouts with either natural or synthetic call stimuli.

Results: Results show that antiphonal vocal response rates were similar between natural phees and synthetic phees that fall within ~1-2 standard deviations [SD] of the population average. However, marmoset response rates were dramatically reduced for synthetic phees outside of ~1-2 SD of the population average. This reduction in vocal response rate for phees falling outside the normal range of variability parallels that seen in our lab's previous perceptual data, where behavioral discrimination thresholds decreased dramatically when synthetic phee stimuli were outside of ~1-2 SD of the population average.

Conclusions: We found a strong agreement between the perceptual category boundary for phee calls and marmosets' willingness to vocally respond to a given phee stimulus. We believe these data are the first to show an association between mechanisms of vocal perception and vocal production in marmoset phee calls, and suggest that marmosets actively assess the behavioral relevance of a perceived vocalization stimulus prior to producing a vocal response.

Podium 12: Immune Regulation and Dysregulation in the Ear: From Animal Models to Humans

3:00 p.m. - 5:00 p.m.

Meeting Room 208ABC, Floor 2

Moderators: Shelby Payne and Bo Hua Hu

Contribution of Embryonic Hematopoiesis and Circulating Monocytes to the Maintenance of Homeostasis in Resident Macrophages in the Postnatal Mouse Cochlea

3:00 p.m. - 3:15 p.m.

Takayuki Okano*¹, Toru Miwa²

¹*Fujita Health University*, ²*Teikyo University Hospital Mizonokuchi*

Background: Embryo-derived tissue-resident macrophages are among the earliest immune cells to appear in multicellular organisms. In mammals, these macrophages arise from specialized precursor cells within the yolk sac during early embryonic development. During organogenesis, they differentiate into tissue-specific macrophage populations, establishing stable, specific relationships with local tissue cells that persist throughout adulthood. These resident macrophages play important roles in maintaining tissue homeostasis, supporting tissue development, and responding to injury and disease. They are essential components alongside the specialized cells of each tissue. For example, in the brain, microglia provide neuroimmune functions as resident macrophages that coexist with neurons. In bone tissue, osteoclasts function as tissue-specific macrophages involved in bone resorption, working in concert with osteoblasts responsible for bone formation.

This auxiliary cell type differs both embryologically and functionally from macrophages derived from hematopoietic stem cells or monocytes. It senses and integrates local and systemic information, providing specialized tissue cells with essential growth factors, nutrient recycling, and waste removal for tissue growth, homeostasis maintenance, and repair. Understanding the proportion and origin of macrophage subpopulations in different tissues is critically important. However, in the inner ear, the specific composition and developmental origin of macrophages remain poorly understood. This study aimed to clarify the origin and developmental dynamics of cochlear macrophages during critical periods for the expression and maturation of auditory function.

Methods: This study utilized three mouse strains: wild-type ICR mice, *Csflr*-deficient mice lacking a receptor essential for monocyte/macrophage differentiation, and *Ms4a3Cre-Rosa tdTomato* transgenic mice enabling fate mapping of monocyte-derived macrophages. Macrophages were labeled using specific markers: *Iba1*, expressed in more mature macrophages, and *CD11b*, a marker primarily associated with the monocyte lineage.

Results: The results revealed significant alteration with age. In adult mice, an increased proportion of monocytes mobilized from the systemic circulation was observed, along with a higher proportion of monocyte-derived macrophages in the cochlea. Conversely, macrophages expressing *Iba1*, but lacking *CD11b* gradually decreased with aging. Fate mapping using *Ms4a3tdT* transgenic mice demonstrated that the proportion of bone marrow-derived macrophages increased with aging, reflecting continuous mobilization of circulating monocytes. Simultaneously, the proportion of yolk sac and fetal liver-derived macrophages contributing to the cochlear macrophage pool steadily decreased over time.

Conclusions: These findings suggest a dynamic shift in the origins of cochlear macrophages from embryonic sources to systemic, bone marrow-derived monocytes in adulthood. Future research should focus on elucidating the specific roles these heterogeneous macrophage populations play in cochlear health, disease states such as infections, autoimmune conditions, metabolic disorders, and their potential as targets for therapeutic interventions aimed at protecting or restoring hearing function.

Mononuclear Phagocytes of the Inner Ear

3:15 p.m. - 3:30 p.m.

Shakila Mahmuda Fatima^{*1}, Muhammad Rahman¹, Md Ibrahim Razu¹, Bryce Hunger¹, Brian Mostaert¹, Shanta Afrin¹, Sharar Haque¹, Nashwaan Khan¹, Zhenshen Zhang¹, Alexander Claussen¹, Marlan Hansen¹

¹*University of Iowa*

Background: Mononuclear phagocytic cells (mononuclear phagocytes) play a significant role in inner ear immunity. Historically, cochlear mononuclear phagocytic cells have been described as either ‘mononuclear phagocytes’ or ‘macrophages’. However, mononuclear phagocytes in most tissues can be further sub-classified as tissue macrophages, dendritic cells, and, in the case of the central nervous system, microglia. Differing embryonic origin and molecular markers distinguish subtypes of mononuclear phagocytes with diverse physiological and pathophysiological functions. The recent discovery of microglia in rat cochleae raises the question of which subtypes of mononuclear phagocytes are present in the cochlea. Here, we identify subtypes of cochlear mononuclear phagocytes, their spatial and morphological organization, and characterize their antigen-presenting function.

Methods: We used three reporter mice in this study: B6.129P2 (Cg)-Cx3cr1^{tm1}Litt/J (CX3CR1+/GFP as reporter for mononuclear phagocytes), C57BL/6-Tmem119^{em2}Gfng/J (TMEM119+/GFP as reporter for microglia), B6.FVB-1700016L21RikTg (Itgax-DTR/EGFP) 57Lan/J (CD11c+/GFP as reporter for dendritic cells). To define the spatial organization of different types of mononuclear phagocytes, 30-µm mid-modiolar sections of cochleae from reporter mice were imaged with confocal microscopy. Using a volumetric segmentation approach, the density of CX3CR1+, TMEM119+, and CD11c+ cells was quantified in specific regions of the cochlea. We additionally quantified the expression of F4/80 (mononuclear phagocytes) and MHCII (marker for specialized antigen-presenting cells) in the cochleae of these reporter mice.

Results: CX3CR1+ mononuclear phagocytic cells are present throughout the cochlea, while TMEM119+ and CD11c+ cells are concentrated around the margin of the peri-lymphatic and endo-lymphatic spaces, and the lateral wall. These three types of cells differ morphologically according to their spatial organization. F4/80 is a sensitive marker for all three cell types with poor specificity for a specific mononuclear phagocyte subtype. We further sub-classified the CX3CR1+ cells based on the level of F4/80 expression. Varied percentages of CX3CR1+, CD11c+, TMEM119+ cells express MHCII.

Conclusions: These data suggest that three major types of mononuclear phagocytic cells exist in the murine cochlea. This study establishes a foundation for further exploration of the functions of heterogeneous subtypes of mononuclear phagocytes in the inner ear.

Single-Cell Transcriptomics Reveals Alterations in the Cochlear Immune Landscape at Hearing Onset in a Murine Model of CMV-Related Congenital Hearing Loss

3:30 p.m. - 3:45 p.m.

Khai Nguyen^{*1}, Daniel R. Romano², Song-Zhe Li², Keiko Hirose²

¹*Washington University in St. Louis School of Medicine*, ²*Washington University School of Medicine*

Background: Cytomegalovirus (CMV) is the most common congenital viral infection and leading cause of acquired sensorineural hearing loss in children; how CMV causes hearing loss is not well understood. It was previously thought that virally induced inflammation contributes to inner ear injury and hearing loss in CMV; however, it is not known which cell types and signaling circuits underlie this process.

Methods: Cochleas from control and CMV+ C57BL/6 mice at P14 (N = 12 mice/group) were dissected, dissociated, and MACS-sorted for CD45+ cells. Single-cell RNA sequencing was performed using the 10X Chromium 3' v4 protocol. Doublets and ambient RNA were removed using DoubletFinder and SoupX, respectively, and data were analyzed using Seurat. Pathway enrichment analysis was done using the bc3net package. Ligand-receptor signaling analysis was performed using NicheNet. Key findings were validated using immunofluorescence.

Results: 7,288 cells were retained for analysis following quality control. CMV+ cochleas showed increased representation of macrophages and T cells compared to controls, which was confirmed by immunofluorescence. In T cells, cytotoxic, interferon-response, and T cell receptor signaling genes were upregulated, while exhaustion genes were downregulated. Macrophages comprised microglial-like and border-associated macrophage-like populations, upregulated antigen presentation and interferon-response genes, and downregulated microglial and lipid-processing genes. Neutrophils upregulated inflammatory cytokines (Ccl6, Il1b, Tnf), and downregulated early-development genes (Elane, Mpo, Mki67). Monocytes upregulated antigen presentation and innate immune receptor genes, and downregulated Ly6c2, Ccr2, and Vcan, suggesting differentiation towards macrophages. Subclustering analyses of T cells and macrophages revealed induction of a cytotoxic/effector T cell state and induction of interferon-responsive and phagocytic macrophages in CMV. NicheNet analysis revealed upregulation of cytokine, chemokine, antigen presentation, and phagocytic receptor-ligand signaling.

Conclusions: Overall, CMV infection shapes the cochlear immune landscape in the developing mouse cochlea, expanding macrophage and T cell populations and inducing cytotoxic, interferon-driven, and antigen-presentation programs. These changes are accompanied by enhanced cytokine, chemokine, and phagocytic signaling, as well as shifts in monocyte and neutrophil states toward more differentiated, inflammatory phenotypes. Together, these findings indicate that CMV promotes a pro-inflammatory, cytotoxic, and antigen-presenting environment

in the cochlea, with activated macrophages and T cells as central mediators, which may contribute not only to viral clearance, but also to cochlear inflammation and tissue injury.

Sympathetic Neurons Modulate Immune Responses to Inner Ear Bacterial Infections

3:45 p.m. - 4:00 p.m.

Katelyn C. Boulanger*¹, Mariah F. Hazlett², Georgia Gunner², Liwen Deng², Isaac M. Chiu², Lisa Goodrich²

¹*Harvard University*, ²*Harvard Medical School*

Background: The sympathetic nervous system regulates tissue physiology across diverse organs, often in response to external stressors. For instance, upon infection, sympathetic neurons modulate immune responses to clear pathogens while limiting damage to healthy tissue. The inner ear receives dense sympathetic innervation, raising the possibility that sympathetic neurons also influence immune activity during inner ear infections such as in bacterial meningitis (BM). BM is caused by bacterial invasion of the meninges and cerebral spinal fluid (CSF), which is contiguous with the perilymph of the inner ear. In addition to disabling headaches, most BM patients experience hearing loss, dizziness, and vertigo, likely caused by spread of bacteria from the CSF. In this study, we tested how sympathetic neuron innervation affects inner ear immune responses in a mouse model of BM.

Methods: We determined the location of inner ear innervating sympathetic neurons (IESNs) by injecting the retrograde tracer cholera toxin subunit B (CTB) into the posterior semicircular canal and examining its distribution in sympathetic ganglia. IESN innervation was characterized by immunohistochemistry for noradrenergic markers, pre-synaptic proteins, and immune cell markers in wholemount preparations. To induce BM in mice, clinical isolates of *E. coli* or *S. pneumoniae*, leading culprits of BM in neonates and adults, were injected into the CSF via the cisterna magna. Control and infected mice were assessed for sickness behavior, vestibular behavior, and acoustic startle response, followed by tissue harvesting and histological analysis. The importance of sympathetic-immune interactions was tested by ablating sympathetic axons via administration of 6-hydroxydopamine (6-OHDA) and by activating sympathetic neurons using the LSL-Gq-DREADD mouse line with chronic administration of ligand clozapine-N-oxide (CNO), followed by induction of BM, behavioral assays, and histological evaluation.

Results: We found that IESNs predominantly arise from the ipsilateral superior cervical ganglion and stellate ganglion and extend abundant axons into the cochlea and vestibular organs, with putative release sites closely apposing resident immune cells. Infected mice displayed poor vestibular behavior and auditory responses that worsened over the first days of infection, mirroring human clinical symptoms. These behavioral deficits correlated with increasing immune infiltration into the inner ear spaces. Furthermore, we found that sympathetic neurons upregulate expression of cFos acutely after BM infection, indicating recent neural activity. Consistent with expression of noradrenaline receptors by most circulating immune cells, we found that chemogenetic activation of sympathetic neurons worsened inner ear behavior in mice with BM. Conversely, sympathetic ablation improved inner ear behavior, indicating a role for noradrenergic signaling in resolution of BM.

Conclusions: Our data suggest sympathetic neurons affect the extent of inflammatory activity in the inner ear during BM infection. Current work aims to dissect molecular and cellular mechanisms of neuroimmune interactions in the inner ear, with potential implications for preserving hearing and vestibular function during infections.

A Stress-Myeloid Inflammatory Axis Drives the Progression of Ménière Disease

4:00 p.m. - 4:15 p.m.

Xiaofei Li^{*1}, Na Zhang², Na Li², Daogong Zhang², Haibo Wang²

¹*Shandong Provincial ENT Hospital, Shandong University,* ²*Shandong Provincial ENT Hospital*

Background: Ménière disease (MD) is a chronic inner ear disorder of unknown etiology. Although an immune-inflammatory link is suspected, the upstream triggers and cellular mechanisms connecting psychosocial stress to inner ear pathology remain poorly defined. This study aimed to investigate the role of stress-induced, myeloid cell-derived inflammation in the progression of MD.

Methods: Perceived stress was evaluated in MD patients and healthy controls using the Perceived Stress Scale-10 (PSS-10). RNA sequencing was employed to characterize the systemic and myeloid cell-specific transcriptional profiles. The association between serum inflammatory cytokine levels and audio-vestibular function was examined in cross-sectional and longitudinal patient cohorts. In vitro experiments on isolated myeloid cells were conducted to explore the regulatory mechanisms of catecholamines and glucocorticoids on inflammatory responses.

Results: MD patients reported significantly higher levels of perceived stress compared to controls. RNA sequencing of peripheral blood revealed a distinct pro-inflammatory transcriptional signature in MD patients. Further analysis identified CD11b⁺ myeloid cells as the primary source of this inflammation, showing significant upregulation of pro-inflammatory genes. Clinically, elevated serum levels of the myeloid-derived cytokine G-CSF were associated with poorer baseline audio-vestibular function, and a longitudinal increase in G-CSF levels correlated with functional deterioration. Mechanistically, MD patients exhibited elevated plasma norepinephrine and increased β 1-adrenergic receptor mRNA in myeloid cells. In vitro, blockade of the β -adrenergic-cAMP pathway attenuated pro-inflammatory cytokine production in these cells. Although systemic cortisol levels remain unchanged, the glucocorticoid receptor sensitivity of myeloid cells in MD patients is altered.

Conclusions: Our study elucidates a potential pathophysiological axis in MD, where perceived psychosocial stress is linked to sympathetic nervous system overactivity. This, in turn, primes circulating myeloid cells for a pro-inflammatory response via the β -adrenergic pathway. The resultant systemic inflammation is closely associated with the severity and progression of audio-vestibular dysfunction. These findings suggest that targeting the stress-neuroendocrine-immune interface may offer novel therapeutic strategies for MD.

Immune Profiling of Human Vestibular Schwannoma Secretions Identifies TNF- α and TWEAK as Cytokines With Synergistic Potential to Impair Hearing

4:15 p.m. - 4:30 p.m.

Sasa Vasilijic*¹, Richard Seist¹, Zhenzhen Yin², Lei Xu², Konstantina M. Stankovic¹

¹Stanford University School of Medicine, ²Edwin L. Steele Laboratories, Massachusetts General Hospital and Harvard Medical School

Background: Vestibular schwannoma (VS) is an intracranial tumor arising from the Schwann cells of the vestibular nerve and is an important cause of sensorineural hearing loss (SNHL) in humans. The mechanisms underlying this SNHL are incompletely understood and currently, there are no drugs FDA approved specifically for VS. This knowledge gap significantly limits the development of effective treatments aimed at preventing, stabilizing, or reversing VS-induced SNHL.

Methods: To identify effector molecules involved in VS-induced SNHL, we analyzed 47 immune-related factors secreted by tumor tissue in over 50 patients with sporadic VS and studied their correlation with preoperative hearing ability and tumor size. The most promising effector molecules were validated in vivo in an anatomically accurate mouse model of VS, and in vitro with mouse fibroblasts (L929) and auditory cell lines representing pro-sensory precursors of hair cells (UB-OC1) and auditory neuroblasts (US-VOT-N33).

Results: We demonstrated that VS-induced SNHL was linked to increased secretion of TNF- α , IL-2R, CD163, eotaxin, and HGF, while larger tumor size was associated with higher levels of TNF- α , TNF-R2, IL-1 α , IFN- α , MIP-1 β , and IL-21 secretion. We identified heterogeneity among VS tumors in their capacity to secrete TNF- α . Tumors with high levels of TNF- α secretion released cytokines and chemokines that significantly correlated with poor hearing (TWEAK and eotaxin) or better hearing (LIF, GRO- α , MIP-1 α , MIP-3 α , and IL-1 α). Among these, TWEAK was notably abundant, with levels exceeding those in normal nerve tissue, elevated in patients with non-serviceable hearing and strongly linked to poor hearing in patients with TNF- α high-secreting tumors. In vivo, we demonstrated that VS-secreted factors reach the inner ear, with elevated TNF- α and TWEAK in the perilymph and blood of tumor-bearing mice with impaired hearing. In vitro, TWEAK amplified TNF- α -mediated cytotoxicity in TNF- α sensitive cells (L929) and auditory cell lines (UB-OC1 and US-VOT-N33) at tumor-secreted concentrations.

Conclusions: This study provides compelling evidence that VS-secreted TNF- α and TWEAK act synergistically to drive tumor-induced SNHL. Targeting the TNF- α /TWEAK axis presents a promising new avenue for preventing VS-induced SNHL.

Defining Immune-Epithelial Dysregulation in Otitis Media With Effusion Through Transcriptional and Cell Surface Proteomic Profiling

4:30 p.m. - 4:45 p.m.

Zhen Jason Qian*¹, Sydney Ramirez¹, Farhoud Faraji¹, Paul Lopez², Arwa Kurabi¹, Allen F. Ryan¹, Shane Crotty²

¹University of California, San Diego, ²La Jolla Institute for Immunology

Background: Otitis media is the most common indication for antibiotics and surgery in children and the leading cause of hearing loss in this population. Most surgical cases are due to otitis media with effusion (OME), defined by culture-negative, mucinous fluid that disrupts sound conduction during critical periods of speech and language development. Despite its prevalence, OME has no effective medical therapy; current management relies on tympanostomy tubes or hearing aids, both costly and imperfect. Progress has been limited by the absence of representative animal models and the scarcity of pediatric middle ear mucosa (MEM) for tissue-level analyses. Here, we present the first single-cell transcriptomic and cell surface proteomic profiles of pediatric MEM, thus establishing a cellular and molecular framework for understanding OME.

Methods: MEM biopsies were obtained from children undergoing tympanostomy tube placement for OME, with age-matched controls from cochlear implant (CI) surgeries. Single-cell suspensions were processed on the 10x Genomics platform for scRNA-seq, with CITE-seq applied to capture surface protein and transcriptomic data in parallel. Spectral flow cytometry of matched aliquots validated epithelial and immune populations, and was further applied to paired middle ear effusions (MEE) and adenoid swabs (AdS) from the same donors to identify accessible biomarkers of mucosal immune states.

Results: CI controls were consistently paucicellular (< 2,000 cells/donor) and displayed resting epithelial, lymphoid, and myeloid population. In contrast, OME samples yielded robust recovery of immune and epithelial cells (< 10,000 cells/donor) with marked expansion and variability of the cellular landscape. The full B cell lineage, including germinal center B cells suggestive of tertiary lymphoid structures (TLS), was observed alongside Tfh and Treg subsets, indicating an organized but dysregulated adaptive response. Secretory epithelial subsets were also expanded in OME, likely reflecting downstream effects of immune dysregulation. Immune profiles from paired MEE and AdS correlated with MEM biology, supporting their potential as accessible biomarkers. Notable inter-patient variability in immune and epithelial composition suggests the existence of distinct OME endotypes with direct implications for clinical translation.

Conclusions: This study provides the first single-cell and surface proteomic profiles of pediatric MEM, showing that OME is driven by immune-epithelial dysregulation. Germinal center B cells, Tfh, and Treg subsets indicate local TLS-like activity, while expansion of secretory epithelial populations highlights downstream dysfunction. Correlated immune profiles across MEM, MEE, and AdS support the feasibility of accessible biomarkers. These findings point toward biologically distinct OME endotypes and lay the groundwork for precision therapies in the leading cause of hearing loss in young children.

Otitis-Prone Children Have Higher Frequencies of Innate Immune Cells and Reduced Cytokine Responses to NTHi and Protein D

3:00 p.m. - 5:00 p.m.

Sharon Clark^{*1}, Elke Seppanen¹, Christian Tjiam², Lauren Bakaletz³, the late Allan Cripps⁴, Harvey Coates⁵, Shyan Vijayasekaran⁶, Peter Richmond⁷, Ruth Thornton²

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Centre for Child Health Research, The University of Western Australia, ³Abigail Wexner Research Institute at Nationwide Children's Hospital, ⁴School of Medicine and Dentistry and Menzies Health Institute Queensland Griffith University, ⁵School of Medicine, The University of Western Australia, Wesfarmers Centre of Vaccines and Infectious Diseases, The Kids Research Institute Australia, ⁶School of Medicine, The University of Western Australia, Perth Children's Hospital, ⁷School of Medicine, The University of Western Australia, Wesfarmers Centre of Vaccines and Infectious Diseases, The Kids Research Institute Australia, Perth Children's Hospital

Background: Chronic and recurrent middle ear infections (otitis media, OM) are a leading cause of children requiring surgery. Recurrent OM is predominantly caused by persistence of nontypeable *Haemophilus influenzae* (NTHi) in biofilms within the middle ear. Children requiring surgical intervention are considered otitis-prone. We have demonstrated previously that otitis-prone children have lower antigen-specific antibody titres and memory B-cell frequencies to NTHi biofilm antigens. We aimed to determine if these findings are due to deficiencies in their cellular immune responses and/or production of antigen-specific cytokines.

Methods: Peripheral blood mononuclear cells (PBMCs) were isolated from otitis-prone (n = 30) and non-otitis-prone (n=15) children. Cell frequencies in PBMCs were assessed for T-cells (CD3+CD19-CD4±CD8±CCR7±CD45RA±), B-cells (CD3-CD19+CD27±IgD±), dendritic cells (DCs; CD3-CD19-CD56-HLA-DR+CD11c±CD123±), monocytes (CD3-CD19-CD56-CD16±CD14±) and natural killer cells (NK; CD3-CD19-CD56+CD16±) using flow cytometry. PBMCs (2x10⁵) were stimulated with NTHi biofilm antigens; Protein D (PD), rsPilA, ChimV4, or live NTHi, and cytokine production was assessed at 24 and 96 hours. Intergroup comparisons were performed using Mann–Whitney U tests.

Results: Compared to non-otitis-prone children, otitis-prone children had higher frequencies of CD8+ T-cells, CD56lo NK-cells, myeloid DCs (mDCs), plasmacytoid DCs (pDCs), and non-classical monocytes, alongside lower frequencies of CD56hi NK-cells (P < 0.044). Cytokine responses were antigen-specific, with rsPilA and ChimV4 rarely inducing a response. At 24 hours post-stimulation with PD or live NTHi, otitis-prone children showed significantly lower levels of IL-8 and IL-10 (P≤0.044). At 96 hours post-stimulation with PD, elevated IL-5 and reduced IL-17 levels were observed in otitis-prone children (P < 0.018). No other significant differences were detected.

Conclusions: Otitis-prone children had higher frequencies of circulating immune cells, potentially reflecting chronic antigenic stimulation or immune dysregulation. Reduced innate cytokines and IL-17 responses may contribute to the lower NTHi-specific memory B cell frequency and antibody titres previously observed. Together these data suggest otitis-prone children have dysregulated NTHi biofilm specific immune responses, which may help explain their increased disease susceptibility. Addressing these antigen-specific immune deficiencies should be a priority in the design for effective otitis media vaccines.

Special Session: Harnessing Human Subcortical and Peripheral Responses to Natural Speech for Translational and Basic Science

Chair: Melissa Polonenko, *University of Minnesota*

Co-Chairs: Ross Maddox, *University of Michigan* & Jonathan Simon, *University of Maryland - College Park*

3:00 p.m. - 5:00 p.m.

Ballroom B, Floor 3

An Introduction to Measuring Subcortical Responses to Continuous Natural Speech

Ross Maddox*¹

¹*University of Michigan*

Individual Abstract: Until recently, the tools for studying the responses of human subcortical nuclei were limited to averaged responses to many repetitions of a short stimulus. This changed a few years ago when new methods started being published by several different labs. This talk will serve as an introduction to and brief history of some of those methods, with a focus on the subcortical temporal response function (TRF), which is akin to the transient-evoked auditory brainstem response (ABR) while not relying on transient stimuli. The basics of subcortical TRF computation will be covered, along with discussion of several variations and their strengths and weaknesses for different applications. The talk will include practical aspects of using the approach in experiments, as well as potential pitfalls related to (for example) stimulus selection and stimulus-EEG synchrony. Higher level considerations about interpreting subcortical TRF results will also be discussed.

Measuring Wide-Band and Multi-Band Auditory Brainstem Responses to Continuous Peak Speech for Future Translation to Evaluating Outcomes in Children

Melissa Polonenko*¹

¹*University of Minnesota*

Individual Abstract: Speech is a dynamic complex sound that is essential for infants and young children to hear during their early years to support spoken language acquisition. Therefore, it is important that we identify hearing loss and other hearing challenges as soon as possible so that we can promptly provide intervention to ensure children receive an adequate representation of the speech that they need to hear. Yet current hearing tests for young children do not use continuous speech and almost a year passes before a child begins to talk or can reliably participate in speech testing. And even then, testing awake children older than 6 months can be challenging and often yields incomplete assessments. But recently the “peak speech” method was developed for deriving auditory brainstem responses (ABRs) to continuous speech (e.g., audiobooks) to investigate underlying neural processing of communication. Now there is a method that uses engaging stimuli for awake children or can be completed if the child naps. This method holds potential for new studies of speech processing and for novel clinical tests that use more ecologically salient and engaging stimuli.

This presentation will highlight two different methods of peaky speech that have future clinical applications to folks with a range of hearing and behavioral abilities. First, peaky speech can be used to measure frequency-specific (multiband) ABRs for the same octave bands that are used to assess hearing and set parameters in hearing aids. We will discuss the method, experimentally-driven optimal parameters, and ways the responses change with hearing loss. Second, we will explore the use of peaky speech in different challenging listening contexts that many folks, including children, experience during a typical day. We have shown ABR changes to narrated speech presented within different signal-to-noise ratios from several simultaneously presented talkers, as well as within different spatial contexts such as co-located or spatially separated talkers.

Both the wide-band and multi-band ABRs to continuous peaky speech hold promise as future objective clinical tests to evaluate speech processing and use of amplification to support speech and language acquisition in children.

Leveraging Subcortical Responses to Natural Speech for Enhancing Hearing Assistive Technology

Florine L. Bachmann*¹, Joshua P. Kulasingham², Kasper Eskelund³, Jens Hjortkjær⁴, Emina Alickovic⁵, Hamish Innes-Brown⁶

¹Eriksholm Research Centre, part of Oticon, ²Automatic Control, Linköping University, ³Oticon A/S, Smørum, ⁴Hearing Systems, Technical University of Denmark, Danish Research Centre for Magnetic Resonance, Copenhagen University Hospital, ⁵Eriksholm Research Centre, Snekkersten, Automatic Control, Linköping University, ⁶Eriksholm Research Centre, Snekkersten, Hearing Systems, Technical University of Denmark

Individual Abstract: Background: Recent methodological advancements have enabled the estimation of the auditory brainstem response (ABR) using continuous speech rather than short, repeated sounds. This response, if sensitive to sound-level changes and peripheral auditory neural processing status, could provide valuable insight into processing of real-life speech in people with hearing difficulties. Especially responses measured while using assistive technology, such as hearing aids, could provide crucial information to further improve solutions for people with hearing difficulties.

Method: To translate the potential of measuring ABRs to continuous speech into applications benefitting people with hearing difficulties, we adopted a step-by-step approach. We (1) investigated whether the method to estimate ABR to continuous speech is sensitive to sound-level changes and auditory peripheral state similar to the click-ABR response. We further quantified ABR to natural speech (2) using speech presented in the sound-field, (3) in the intended target group – people with hearing difficulties, (4) with amplification provided through insert earphones, and combining these elements by (5) presenting people with hearing difficulties with speech in the sound-field, amplified through standard hearing devices.

Results: Our results show consistent neural response peaks akin to click-ABR wave V in the ABR derived from continuous natural speech stimulation. We demonstrate that, like traditional click-ABR wave V, ABR responses to continuous speech are sensitive to sound-level and auditory peripheral changes. We further quantified ABR responses to continuous speech in individuals with hearing difficulties, with sound provided in the sound-field and amplified with standard hearing aids.

Discussion: Assessing ABR responses to natural speech offers high ecological validity and may inform the development and fitting of hearing assistive devices. Evaluating hearing technology aiming to maximize speech understanding using a measure of objective neural brainstem processing of real-life speech - the signal it is primarily optimized for - could help identify new directions in hearing aid development. Moreover, it holds the potential for tailored fitting of existing hearing technology to individuals' listening needs. This approach may further pave the way towards hearing state assessments and technological adjustments seamlessly integrated into daily life in the future.

Mapping Subcortical Contributions to Speech-In-Noise Perception Using Chirped Speech (Cheech)

Kelsey Mankel*¹

¹*University of Memphis*

Individual Abstract: Speech recognition abilities, particularly in noisy environments, varies significantly across individuals, even among those with audiometrically normal hearing thresholds. One likely contributor to this variability lies in how efficiently auditory signals are encoded along the auditory pathway, particularly in brainstem structures where timing and precision are essential for parsing speech. While standard auditory evoked potential protocols have provided some insight into subcortical processing, they typically rely on brief, isolated stimuli that do not reflect the complexity of real-world speech. As a result, they often fall short in predicting individual listening performance, especially in more naturalistic, noisy settings. Recent advances in stimulus design and neural recording methods now allow researchers to capture brain responses to more ecologically valid speech signals.

One of these approaches includes a novel technique known as chirped speech (Cheech) in which continuous speech is embedded with brief frequency sweeps designed to rapidly (< 10 minutes) and simultaneously elicit robust neural responses across the auditory hierarchy from brainstem (e.g., ABRs) through cortical responses (e.g., late-latency responses; LLRs). This talk will highlight recent applications of Cheech that examine the relationship between individual differences in speech perception—particularly speech-in-noise performance—and subcortical processing. We used Cheech-modified AzBio sentences in quiet and multi-talker babble to reveal how early auditory encoding supports speech recognition abilities across young and middle-aged adults. Other Cheech-based paradigms using short story narratives have additionally offered unprecedented insight into the connections between lower-level auditory evoked potentials and higher-level processes such as comprehension, selective attention, and listening effort.

Across these studies, subcortical encoding emerged as a key predictor of behavioral speech listening performance, with earlier and more robust ABRs associated with better speech-in-noise recognition, comprehension, and lower subjective listening effort. Collectively, these results suggest a key role of the brainstem in successful speech perception. Cheech also demonstrates promise as a potential clinical tool; its ability to evaluate subcortical processing using ecologically valid stimuli makes Cheech well-suited for detecting subtle auditory-perceptual deficits, tracking age-related changes, and informing audiologic interventions in real-world populations.

Computing the FFR to Naturalistic Speech: Neural Sources, Effects of Selective Attention and Influence of Musical Training

Tobias Reichenbach*¹

¹*Friedrich-Alexander-University Erlangen-Nürnberg*

Individual Abstract: Voiced parts of speech exhibit a distinctive spectral structure composed of the fundamental frequency (f_0) and its higher harmonics. This structure elicits a neural response at f_0 and, to a lesser extent, at the higher harmonics. Due to its similarity to the frequency-following response (FFR) to pure tones, this neural response is often referred to as the speech-FFR. Traditionally, it has been measured by repeatedly playing short speech tokens—such as single syllables—a few hundred times to participants and averaging the resulting neural recordings. However, natural variations in the fundamental frequency of continuous speech hinder the averaging of responses to short segments.

Here, we present an approach for measuring the speech-FFR to naturalistic, continuous speech. We extracted a waveform from the speech signal that isolates a narrow frequency band around f_0 . This f_0 waveform could then be related to neural activity using cross-correlation or temporal response functions (TRFs). Neural data was recorded non-invasively using electroencephalography (EEG) or magnetoencephalography (MEG).

We identified two primary components of the speech-FFR: a subcortical component with a latency of approximately 9 ms, likely originating in the inferior colliculus, and a cortical component occurring around 30 ms, originating in the auditory cortex. While EEG recordings were dominated by the subcortical source, MEG—due to its limited sensitivity to deep brain structures—primarily captured the cortical component.

The ability to measure the speech-FFR during continuous speech opens the door to exploring its role in speech processing. As a first step, we examined whether the speech-FFR is modulated by selective attention to one of two competing talkers. We found that both the subcortical and, more prominently, the cortical components are enhanced when the corresponding voice is attended rather than ignored. This demonstrates that the speech-FFR is influenced by top-down feedback from higher cognitive processes. We also investigated whether musical training affects the cortical component of the speech-FFR or its attentional modulation but found no significant influence.

In summary, our work provides a method for assessing the speech-FFR in more ecologically valid settings involving continuous speech. This approach paves the way for further research into

how the speech-FFR is shaped by higher-level cognitive processes and may eventually lead to clinical applications, such as diagnosing or treating speech-in-noise comprehension difficulties.

Attention Effects on Subcortical Responses to Continuous Speech

Zilong Xie*¹

¹*Florida State University*

Individual Abstract: A growing body of research has used continuous speech, such as audiobook stories, to non-invasively assess the encoding of natural sounds at the subcortical levels of the auditory system. These studies have involved tasks requiring varying degrees of attention to speech stimuli, ranging from actively listening and responding to questions about the stimuli to ignoring them while focusing on non-auditory tasks. This raises important questions about whether and how attention affects the subcortical encoding of continuous speech.

To investigate this, we recently conducted a study (Xie, 2025, *Journal of Neurophysiology*) using an audiovisual dual-task paradigm designed to divert attention from the speech stimuli. In this study, participants performed a primary visual memory task with variable cognitive loads—either low or high—while concurrently listening to an audiobook in quiet conditions as a secondary task. These dual-task conditions were compared to a control condition in which participants focused solely on the speech stimuli. Young adults with normal hearing participated in all three conditions, during which we recorded their EEG signals. Temporal response functions (TRFs) were used to estimate subcortical responses to continuous speech by predicting EEG responses from the speech stimuli processed through an auditory nerve model.

The TRFs from all participants across conditions exhibited a pronounced peak around 8 ms, akin to the wave V peak seen in auditory brainstem responses, suggesting that these responses to continuous speech originate from subcortical structures. Importantly, the latencies and amplitudes of this peak did not significantly differ across conditions. The prediction accuracies of the TRF models were also consistent across conditions.

These results indicate that diverting attention through different sensory modalities may not significantly impact the subcortical encoding of continuous speech in quiet conditions. Our ongoing research seeks to explore further the effects of attention on the subcortical processing of continuous speech in noise conditions. Given the variety of tasks examined, these findings have significant implications for future fundamental and translational research on the subcortical encoding of continuous speech.

Separating Cortical From Subcortical Responses to Continuous Speech Using Simultaneous EEG and MEG

Karl Lerud*¹, Charlie Fisher¹, Vrishab Commuri¹, Samira Anderson¹, Jonathan Simon²

¹*University of Maryland - College Park*, ²*Institute for Systems Research, University of Maryland*

Individual Abstract: Early auditory responses such as the auditory brainstem response (ABR, 0 – 10 ms) and the middle latency response (MLR, 10 – 50 ms) are both well-studied and clinically and scientifically important. Typically, a repeated transient auditory stimulus (e.g., click) is used

to elicit an evoked cascade of peaks and troughs at predictable latencies, recorded through electroencephalography (EEG).

In recent years ABR- and MLR-like responses have been recorded in a continuous rather than discrete fashion, using a temporal response function (TRF) paradigm, as opposed to the evoked paradigm. Here, a stimulus such as long-duration continuous speech is used instead of repeated transients, and is also used to generate multiple regressors (feature-based predictors) to use for deconvolution of multiple feature-based TRFs. The use of TRF methodology here has similarities, as well as differences and unique challenges, with the analogous paradigm for deriving cortical responses at the time scales of hundreds of milliseconds. Most importantly, the use of continuous speech for ABR- and MLR-like responses confers many additional benefits, including the ability to record concurrent responses from multiple cortical areas, including general auditory, speech-specific, language-centric, and even higher order areas.

Research thus far has demonstrated that prominent and early responses such as wave V of the auditory brainstem TRF have strikingly similar characteristics to their evoked counterparts, while at the same time revealing additional properties, such as variable latencies and amplitudes as a function of stimulus parameters. The same appears to be true for the several peaks making up the middle latency TRF, which is expected to contain both cortical and subcortical contributions, based on its strong similarity to the MLR.

Here we record concurrent EEG and magnetoencephalography (MEG) responses from young normal-hearing adults listening to a narrated audiobook (both in isolation and with masking from competing speakers). Neural source-localization techniques identify the subcortical and cortical sources of different peaks of: auditory brainstem TRFs, middle latency TRFs, and others, using concurrent MEG and EEG responses (and also only EEG and MEG separately). Since EEG is sensitive to both subcortical and cortical sources, but MEG only to cortical sources, the combination offers a unique opportunity to disentangle the source contributions. Here we show evidence for separable cortical and subcortical contributions to the middle latency TRF, with the relative cortical contribution increasing with increasing peak latency, but not so for the auditory brainstem TRF, which is entirely subcortical.

Mini Podium 2: Ear Repair Mechanisms: Unlocking Sensory Regeneration Across Vertebrates

4:15 p.m. - 5:15 p.m.

Meeting Room 209ABC, Floor 2

Moderators: Esther Fousert and Nesrine Benkafadar

Activation of Yap Signaling for Hair Cell Regeneration in the Adult Mouse Utricle

4:15 p.m. - 4:30 p.m.

Kimberly Ramirez*¹, Juan Llamas¹, Ksenia Gnedeva¹

¹*Keck School of Medicine University of Southern California*

Background: Vestibular hair cells (HCs) are vulnerable to damage from aging, ototoxic agents, genetic mutations, and head or blast trauma. Limited HC regeneration has been observed in mammalian models, arising from supporting cells (SCs) through direct transdifferentiation rather than cell cycle re-entry, which depletes the SC pool. The Hippo pathway is a key regulator of cell proliferation, controlling nuclear translocation of yes-associated protein 1 (YAP). Previous in vitro studies have established that SCs from adult mouse utricles can proliferate following Hippo pathway inactivation or transduction with a constitutively active version of YAP. However, in vivo approaches to transiently activate YAP have not been established. Here, we developed viral vector– and small molecule– based techniques to induce SC cycle re-entry in the adult utricle in vivo and assess its effects on HC regeneration.

Methods: Posterior semicircular canal injections were performed in young-adult CD1 mice to deliver either recombinant adeno-associated virus encoding a constitutively active version of YAP (YAP5SA) or small molecule Hippo inhibitors for YAP activation. 5-ethynyl-2'-deoxyuridine (EdU) was administered twice daily for four days starting one day post-injection. Otic capsules were harvested five days after injection, fixed in 4% paraformaldehyde, and utricles dissected for staining with EdU, SOX2, and BRN3C. mScarlet served as a marker of viral infection. Contralateral ears served as controls.

Results: YAP activation induced cell cycle re-entry in a subset of utricular SCs in vivo, but the effect was age-dependent with a larger percent of SCs reentering the cell cycle in younger mice. At five days post-injection, few proliferating cells were retained. Minimal viral spread was detected in contralateral ears, which showed no evidence of proliferation.

Conclusions: Our findings demonstrate that constitutively active YAP can induce SC cycle re-entry in the adult utricle in vivo, though the effect is age-dependent and transient. In contrast to in vitro studies where proliferating SCs could be maintained across all ages tested, including mice up to 12 months of age, YAP-mediated control in vivo appears more constrained and may depend on dose and tissue environment. Future work will determine whether SCs that re-enter the cycle contribute to HC regeneration. This model provides a platform for investigating the mechanisms governing SC fate and Hippo/YAP signaling in the adult inner ear.

Characterization of the Transcriptomic Response of Avian Utricular Supporting Cells to Sisomicin-Induced Hair Cell Death

4:30 p.m. - 4:45 p.m.

Ishwar Hosamani*¹, Stefan Heller²

¹*Stanford University School of Medicine*, ²*Stanford University School of Medicine, Institute for Stem Cell Biology and Regenerative Medicine*

Background: Vestibular hair cells (HCs) in the inner ear are critical mechanosensory receptors that detect head movements and maintain balance. Loss or damage to these cells leads to balance dysfunction, dizziness, and increased fall risk, greatly affecting quality of life. Unlike mammals, non-mammalian vertebrates such as birds can robustly regenerate mature vestibular HCs post-damage and continuously replenish them through supporting cell (SC) mitotic turnover. These SCs act as facultative stem cells, enabling lifelong HC regeneration. Our research aims to

decipher the molecular mechanisms activated in SCs following HC death and to compare regenerative pathways between vertebrate and non-vertebrates.

Methods: Chicken utricle HCs were ablated by posterior semicircular canal injection of sisomicin (50 mg/ml) at post-hatch day 7 (P7). Single-cell RNA sequencing (scRNA-seq) of the damaged sensory epithelium is conducted at 6, 24, 48 and 96 hours, and at 7 and 14 days post-sisomicin treatment (PST). The data across all time points will be integrated to understand the temporal order and coordination of molecular events from HC death to regeneration and identify early SC response genes versus late HC differentiation markers. We will validate potential molecular markers for SC activation, progenitor cell states, and HC differentiation by in situ HCR and immunofluorescence.

Results: We have previously established that significant utricular HC death occurs at 6 hours PST, with complete loss by 12 hours. The 6-hour PST data will yield the transcriptomic signature of dying HCs and responding SCs, whereas data from the 24- and 48-hour PST timepoints will capture transcriptomic changes in SCs as they respond to HC death and initiate mitotic regeneration. The 96-hour PST timepoint data will characterize nascent HC identities and newly divided SCs. Finally, 7- and 14-day PST timepoints will reveal the transcriptomic identities of regenerated HCs at early and late maturation stages.

Conclusions: This temporal single-cell transcriptomic analysis will reveal gene expression dynamics underlying avian vestibular HC regeneration. Additionally, by comparing transcriptomes of regenerated HCs at 7- and 14-days PST with undamaged avian utricle HCs, we will identify maturation related gene-expression differences, uncover molecular signatures unique to regenerated versus native HCs, and highlight pathways incompletely reactivated or uniquely altered during regeneration. Furthermore, avian utricle regeneration data will be compared with inner ear developmental datasets from various timepoints and existing basilar papilla (BP) datasets, to uncover shared and distinct pathways regulating SC behavior during development and HC damage in the BP. Finally, we will contrast the avian datasets with steady-state and post-damage SC transcriptomes of mammalian utricles to identify fundamental molecular differences governing regenerative capacity. This comprehensive cross-species comparison aims to highlight key signaling pathways that differ between species during HC death, SC activation, HC regeneration and maturation, thus offering potential targets for regenerative therapies in mammals, including humans.

Mechanical Stress Regulates Age Dependent Differentiation and Regeneration Patterns in the Mammalian Vestibular System

4:45 p.m. - 5:00 p.m.

Shahar Kasirer^{*1}, Michal Shraga¹, Tim Dullweber², Olga Loza¹, David Sprinzak¹

¹Tel Aviv University, ²University of Basel

Background: In the vestibular system, lost hair cells (HC) can be regenerated by the trans-differentiation of nearby supporting cells (SC). For mammals, this regenerative capacity declines rapidly with age. In recent years, significant efforts have been directed towards developing regeneration promoting treatments, with encouraging, but limited success. These efforts are hampered by the lack of mechanistic understanding of regeneration dynamics. It is known that HC differentiation is regulated by Notch-mediated lateral inhibition. In addition, recent studies

indicate that mechanical stress might have a regulatory role during regeneration. However, it is yet unclear if HC regeneration is indeed regulated by mechanical forces, and if so, how it is coordinated with Notch mediated lateral inhibition.

Methods: We developed a live imaging assay for mouse utricle explants. In this assay we can monitor cell morphology and differentiation dynamics using mice expressing ZO1-EGFP (marking apical boundaries) and Atoh1-mCherry (marking HC differentiation). To track the local regeneration dynamics, we perform live imaging following single HC laser ablation. The same laser is used to make circular cuts in the tissue, and compare the age dependent viscoelastic response, to measure the relative mechanical stress. In addition, we perturb both Notch signaling (via Notch inhibition) and mechanical stress (via Rho-kinase inhibition) to study their regulatory roles. Finally, relying on insights from our experimental results, we formulate a mathematical model for utricle development and regeneration, that includes biochemical signaling and mechanical forces. Our model predictions are then validated via quantitative comparison with experiments.

Results: Quantitative analysis of differentiation dynamics shows unexpected age dependent deviations from the classical lateral inhibition model: While at earlier stages (E17.5) most differentiating HC do not have any HC neighbors, at more advanced age (P0) most HC differentiate next to existing HC. This is true both for spontaneous HC differentiation and for SC trans-differentiation in response to HC ablation. Our measurements indicate the weakening of mechanical stress in the tissue over time. Rho kinase inhibition relieves mechanical stress in the tissue and eliminates the age dependent differences in differentiation dynamics, demonstrating the regulatory role of mechanical stress. We formulate a revised mathematical model of HC differentiation where lateral inhibition is coupled to mechanical stress. This model predicts correctly the observed differentiation and trans-differentiation patterns as well as the response to Notch and Rho-kinase inhibition.

Conclusions: Overall, our experimental results highlight the regulatory role of mechanical stress in utricular HC differentiation and regeneration. Combining these results with mathematical models strongly supports a model in which this regulatory role is implemented through the mechano-regulation of Notch-mediated lateral inhibition. These results also suggest that the decline in regenerative capacity in the vestibule may be associated with changes in its mechanical properties with age, in a way that affects local differentiation dynamics.

Islet-1a Orchestrates Gene Expression Dynamics by Reducing Chromatin Accessibility During Zebrafish Regeneration

5:00 p.m. - 5:15 p.m.

Tatjana Piotrowski^{*1}, Paloma Meneses-Giles¹, Kaelan Brennan¹, Nhung T T Tran¹, Aurélie Hintermann¹, Mark Lush¹

¹*Stowers Institute for Medical Research*

Background: Isl1 is a transcription factor critical in proliferation, differentiation, and survival of progenitor cells in multiple tissues. Its overexpression in concert with other transcription factors aids in mouse hair cell production during regeneration, but its mechanisms of action are poorly understood. Zebrafish lateral line hair cells are homologous to mammalian hair cells but robustly

regenerate throughout life. Our published scRNA-seq analyses of regenerating lateral line organs revealed that during homeostasis *isl1a* is expressed in central support cells that give rise to hair cell progenitors, as well as in young hair cells.

Methods: We show that *isl1a* is downregulated immediately after hair cell death but is re-expressed at 3hr after neomycin treatment. We generated a full gene *isl1a* deletion mutant that is characterized by enlarged sensory organs due to increased number of support cells and hair cells.

Results: Edu incorporation assays and timelapse analyses demonstrated that both the stem cells and the progenitor cells overproliferate. As hair cell numbers disproportionately increase *isl1a* has two functions: 1) it inhibits proliferation but 2) also modulates hair cell specification. To elucidate the molecular mechanisms underlying these phenotypes we performed RNA-seq and in situ hybridization analyses which demonstrated that immediate response and regeneration-specific genes that in siblings are only immediately activated after hair cell death remain upregulated in the mutants. ATAC-Seq analyses of mutants and siblings revealed that *isl1a* reduces chromatin accessibility of target genes that contain *Isl1* motifs suggesting that *isl1a* directly represses a set of regeneration genes during homeostasis. As hair cells die, *isl1a* is downregulated, leading to the activation of the regeneration response which is again restrained when *isl1a* is re-activated a few hours later.

Conclusions: Combined our results show that *isl1a* plays a crucial role in orchestrating the gene expression dynamics during regeneration.

Sunday, February 8, 2026

Podium 3: Benchtop to Bedside and Back: Clinical Otolaryngology and Pathology

8:00 a.m. - 10:00 a.m.

Ballroom B, Floor 3

Moderators: Daniel Sun and Nicole Jiam

Biomarkers of Neuroplasticity in the Perilymph in Prelingual Deafness

8:00 a.m. - 8:15 a.m.

Monika Matusiak*¹, Anna Konopka², Monika Ołdak¹, Dominika Oziębło¹, Henryk Skarżyński¹

¹*Institute of Physiology and Pathology of Hearing*, ²*PORT Łukasiewicz Research Network*,

Background: Congenital deafness treatment with cochlear implantation (CI) is a standard therapy with, in overall, very good results, however, in some cases implanted children do not reach the age norm in verbal communication. Molecular biomarkers would facilitate the clinical management of deaf born children, helping in predicting the level of linguistic proficiency a child would achieve after CI. Having known, from our previous studies, that the serum level of matrix metalloprotease 9 (MMP-9) has been identified as a biomarker of neuroplasticity in prelingual deafness it is of great interest to investigate the MMP-9 also in the perilymph, as well as look for another biomarkers in the perilymph. Inner ear liquid biopsy would also help in understanding the molecular environment of the diseased organ in congenital deafness cases.

The objective of the study is to investigate the perilymph proteome, including MMP-9, and blood serum proteome, and to see their relation to auditory development after CI.

We have collected 6 pairs of samples perilymph/blood during the surgical procedure of CI from prelingually deaf children in Institute of Physiology and Pathology of Hearing, Warsaw. The perilymph was sampled by using glass capillary via the round window just before the array insertion.

Methods: Using Mass Spectrometry (MS) protein quality analysis was performed in each perilymph/blood sample. MMP-9 was assessed by Enzyme-Linked Immunosorbent Assay (ELISA). Auditory development of implanted children was assessed by using LittleARs Questionnaire (LEAQ).

Results: We have detected 1471 perilymph proteins, including MMP-9, out of which 441 were detected only in perilymph and 43 were detected only in blood plasma. Preliminary analyses of correlations between perilymph proteomic profiles, blood plasma and auditory development were performed.

Conclusions: There are correlations between proteome content of the perilymph and auditory development outcomes of cochlear implantation in prelingual cases.

Can Impedance Predict Intracochlear Neo-Ossification and Fibrosis in Cochlear Implant Recipients?

8:15 a.m. - 8:30 a.m.

Nicole E. Tomassi*¹, Abbie Hall¹, Caylin McCallick¹, Alexander Geerardyn¹, Julie G. Arenberg¹, Alicia M. Quesnel¹

¹*Mass. Eye and Ear, Harvard Medical School*

Background: Foreign body responses are a common result of cochlear implantation. Although surgical techniques have changed over the years in attempts to reduce insertion trauma, neo-ossification and fibrosis around the electrode array is still commonly observed. Historically, neo-ossification and fibrosis have been thought to affect the resistivity of electrodes, measured clinically as the level of impedance. Since neo-ossification and fibrosis cannot be easily visualized and quantified in living humans, the relationship between these foreign body responses and cochlear implant impedance levels has been limited to animal and tissue-engineered models. However, post-mortem temporal bone specimens enable high resolution 3-dimensional mapping and quantification of intracochlear fibrosis and neo-ossification, allowing the investigation of these relationships in human cochlear implant recipients. The purpose of this study was to relate neo-ossification and fibrosis volume adjacent to individual electrodes in human implanted cochleae to impedance levels measured during life.

Methods: Human temporal bones from cochlear implant users were histologically processed, sectioned, and digitized. 3D reconstructions of the cochleae and electrodes were generated. The anatomical regions of the cochlea including the scala tympani, scala vestibuli, and scala media (when applicable), as well as the electrode array and areas of neo-ossification and fibrosis, were digitally segmented. The individual electrode locations were mapped using the 3D electrode reconstruction and manufacturer reported intra-electrode distances. The last measured impedance before death was extracted from the clinical records of each temporal bone donor. The amount of neo-ossification and fibrosis was compared to impedance levels using Pearson's correlation coefficient.

Results: We expect a total of 16 donors to be represented in this study. Within these clinical records, impedance levels were higher at the base of the array (likely due to insertional trauma) in 14 donors. Preliminary results in seven completely analyzed temporal bone specimens indicate a direct relationship between neo-ossification and impedance at the group level such that more bone deposition was positively correlated with higher impedance levels ($p < 0.001$, $r = 0.484$). The relationship between neo-ossification and impedance at the individual level varies. In the preliminary dataset, no significant relationship was observed between fibrosis and impedance.

Conclusions: Thus far, a positive relationship has been observed between neo-ossification and impedance at the group level. This approach bridges otopathology and clinical outcomes in humans by quantifying these foreign body responses with high precision and relating them to impedance levels of each electrode.

Distribution Patterns of Cochlear Endosteal Invasion in Otosclerosis

8:30 a.m. - 8:45 a.m.

Tomotaka Shimura¹, Nevra Keskin Yilmaz¹, Sebahattin Cureoglu¹, Meredith Adams¹, Rafael da Costa Monsanto^{*1}

¹*The University of Minnesota*

Background: Most cases of otosclerosis involve bone remodeling localized to the fissula ante fenestram. In a subset of cases, however, the pathological process extends to the cochlear endosteum, resulting in fibrosis, atrophy, or ossification of the cochlear lateral wall. Owing to the limited resolution of current imaging modalities in delineating intracochlear alterations and the precise interactions between osseous and membranous structures, the spatial distribution and morphological patterns of these lesions remain insufficiently defined. The objective of the study was to characterize the frequency, severity, and site-specific distribution of cochlear endosteal invasion in otosclerosis from a pathological perspective.

Methods: Archival histological slides of human temporal bones from donors with otosclerosis from the Paparella Otopathology and Ear Pathogenesis Laboratory collection (University of Minnesota) were examined. Endosteal invasion was defined as direct disruption of the cochlear endosteum and classified into three grades. Grade I was defined as partial contact with the endosteum accompanied by mild surface irregularity, without spiral ligament hyalinization or stria vascularis atrophy. Grade II was characterized by mild pathological changes of the lateral cochlear wall, whereas Grade III encompassed moderate to severe alterations of the lateral wall and/or the presence of intraluminal pathology. Results were statistically analyzed using chi-square tests.

Results: Among 207 ears from 119 donors, cochlear endosteal invasion was identified in 37 ears (17.9%). The distribution of invasion grades was: Grade I, 21.6%; Grade II, 43.2%; and Grade III, 35.1%. Multifocal invasion was significantly associated with higher-grade lesions ($p < 0.0001$). The lower basal turn had the lowest overall frequency of lesions, with an invasion rate of 53.9% compared to other turns. Notably, however, all 7 cases with basal turn involvement demonstrated severe (Grade III) endosteal invasion.

Conclusions: Cochlear endosteal invasion was present in 17.9% of otosclerosis cases and showed site- and grade-specific variability, with the lower basal turn most often affected by severe lesions. This classification system highlights distinct spatial and morphological patterns that may serve as a standardized framework for future clinicopathological studies and could help guide both the timing of medical treatment (e.g., bisphosphonates) and surgical planning.

Hypoxia-Driven Metabolic Reprogramming in Vestibular Schwannoma

8:45 a.m. - 9:00 a.m.

Mikhail Marasigan^{*1}, Olena Bracho¹, Bryan Souza¹, Diane Jung¹, Olivia Kalmanson¹, Michael Ivan¹, Carolina Benjamin¹, Vivek Kanamuri¹, Simon Angeli¹, Fred Telischi¹, Pei-Ciao Tang¹, Daniela Frasca¹

¹*Miller School of Medicine, University of Miami*

Background: Cancer cells adapt their metabolism to sustain proliferation and survive under nutrient limitation and hypoxia, promoting growth and therapy resistance. Vestibular

schwannomas (VS) are benign intracranial tumors of the vestibulocochlear nerve that often cause hearing loss, dizziness, and neurological deficits. Although VS express hypoxia-related factors, their metabolic pathways remain poorly defined. Elucidating these mechanisms may uncover metabolic vulnerabilities that can be targeted to improve clinical outcomes for VS patients.

Methods: Primary VS cell cultures were established from patient tumors. In parallel, wild-type (WT) and NF2-mutant Schwann cells (SCs) were generated from human induced pluripotent stem cells. Cells were maintained in proliferative Schwann media at 37°C, 5% CO₂, under oxygen tensions of 1%, 5%, or 21% O₂. After seeding (2,500 cells/well, 384-well CellBIND plates) and cell adherence, media was refreshed and metabolic assays (CellTiter-Glo, RealTime-Glo MT, Lactate-Glo, Glucose-Glo, Glutamine-Glo) were performed at 72 hours. Luminescence was measured, and data analyzed by nonparametric analysis of variance.

Results: ATP levels correlated with viability measurements, with the highest levels observed at 21% O₂, indicating mitochondrial respiration as the predominant means of energy production when oxygen is readily available. All cell types utilized glucose and secreted lactate (byproduct of glycolysis), though VS exhibited the lowest levels overall. Notably, VS cells increased glutamine consumption at 1% and 5% O₂ compared to 21% O₂, suggesting hypoxia-dependent reliance on glutamine metabolism.

Conclusions: VS, WT, and NF2-mutant SCs display distinct metabolic profiles shaped by oxygen availability. Our pilot study shows VS demonstrate limited glycolysis but enhanced glutamine utilization under hypoxia, pointing to metabolic dependencies that may be exploited therapeutically. Targeting these adaptive pathways could inform novel strategies for VS control.

Intraoperative Electrically Evoked Stapedial Reflex Thresholds (eSRT) for Cochlear Implant Programming: A Postoperative Validation Study

9:00 a.m. - 9:15 a.m.

Eric Cunningham^{*1}, Isabelle J Chau¹, Rivka Bornstein², Regina Albinus², Pal Sudhirkumar Shah¹, Katrina Stidham²

¹New York Medical College, ²Westchester Medical Center

Background: Cochlear implants (CIs) restore hearing in severe-to-profound sensorineural loss by delivering electrical stimulation to the auditory nerve. Programming requires determining the lower (T-level) and upper (C-level) stimulation limits, which define the electrical dynamic range. In patients unable to provide reliable behavioral feedback, the electrically evoked stapedial reflex threshold (eSRT) can approximate C-levels and improve programming accuracy. The eSRT correlates with behavioral C-levels, but postoperative testing is limited by middle ear pathology, patient intolerance, or low clinical adoption. Intraoperative eSRT may overcome these barriers and offer an initial programming reference, but volatile anesthetics can suppress the reflex. Whether intraoperative eSRT predicts early postoperative values, particularly at middle and apical electrodes, remains unclear.

Methods: In this prospective cohort study, CI recipients underwent routine intraoperative eSRT testing with Cochlear Americas devices. Exclusion criteria included absence or disruption of the stapedial tendon, anatomic barriers, medical instability, or volatile anesthetic exposure within 10 minutes of testing. A single surgeon measured intraoperative eSRTs visually with volatile

anesthetics withheld. Postoperative eSRTs were measured ~1 month later. Response rates were compared via chi-squared tests with Benjamini-Hochberg correction. Threshold changes were analyzed with paired t-tests, and intra- vs. postoperative correlations assessed using Spearman's rank.

Results: Eighty-four cochlear implants in 77 patients met inclusion criteria, with intraoperative eSRT attempted in 72 ears and postoperative testing in 50. Intraoperative response rates were significantly higher than postoperative rates overall (83.1% vs. 70.9%, $p = 0.0005$), with the highest rates at middle and apical electrodes. Median intraoperative thresholds were significantly higher than postoperative thresholds (21.2 vs. 15.6, $p < 0.0001$), but no significant correlations were observed between the two time points.

Conclusions: Intraoperative eSRT was feasible and yielded high response rates, particularly at apical and middle electrodes. However, intraoperative thresholds did not correlate with early postoperative values and cannot currently predict programming levels. These findings support the utility of intraoperative eSRT as a qualitative intraoperative tool, but further refinement is needed to improve its predictive value for postoperative cochlear implant programming.

Linking Data-Driven Audiometric Phenotypes to Speech Perception in Quiet and Noise

9:15 a.m. - 9:30 a.m.

Varsha Athreya*¹, Jwala Rejimon², Malcolm Slaney¹, Matthew Fitzgerald²

¹Stanford University, ²Stanford University School of Medicine

Background: The pure-tone audiogram (250–8000 Hz) remains the clinical gold standard for assessing hearing, yet it only partially captures suprathreshold deficits. Here, we apply unsupervised, data-driven profiling to derive phenotypes that reflect both audibility loss and suprathreshold performance (word recognition in quiet and speech-in-noise). This approach refines hearing-loss classifications by capturing natural variability in audiogram shape and severity, and by characterizing speech outcomes within each cluster to support precision audiology. In addition, we will identify specific diseases within each cluster (e.g., Ménière's disease, vestibular schwannoma) to probe disorder-linked audiometric and speech-perception signatures. Finally, we will link these phenotypes to real-world device behavior, specifically the adoption of hearing aids and cochlear implants, to inform targeted fitting strategies and treatment selection.

Methods: We conducted a retrospective analysis of audiological records collected at our academic medical center since 2006, excluding cases with conductive or mixed hearing loss (air-bone gap ≥ 10 dB HL). Using K-means clustering (scikit-learn, Python) on 66,956 unique pure-tone audiograms measured at eight frequencies (250–8000 Hz), we derived distinct, data-driven audiometric phenotypes. To relate these phenotypes to functional hearing, we examined speech-perception performance using Word Recognition in Quiet (WRQ) and QuickSIN (speech-in-noise) measures. Within each cluster, we selected individuals with Ménière's disease and vestibular schwannoma to evaluate relative prevalence and to describe diagnosis-linked speech-perception profiles. Finally, we linked cluster membership and diagnosis to real-world device behavior of hearing-aid and cochlear-implant adoption.

Results: K-means clustering uncovered a set of distinct audiometric phenotypes, highlighting diverse configurations and severities across the cohort. Hearing loss increased with age, most prominently at higher frequencies. Despite similar pure-tone averages, audiograms show age-dependent variability in configuration. As hearing loss increases, the likelihood of sub-optimal word recognition in quiet increases, however, most patients display excellent performance. In contrast, even small amounts of hearing loss, particularly in higher frequencies, worsens speech-in-noise performance. In addition, SPIN performance showed greater individual variability than WRQ scores, demonstrating sizable differences in suprathreshold processing under challenging listening conditions. Ménière's disease tends to show rising configurations in younger patients, possibly linked to recent diagnosis, and flat or sloping losses in older patients. In contrast, vestibular schwannomas more often manifest as sloping losses of variable severity.

Conclusions: These findings emphasize that audiometric thresholds alone cannot capture the full complexity of hearing impairment. Clustering revealed distinct phenotypes tied to age and diagnosis, while differences between word recognition in quiet and speech-in-noise performance suggest that the latter is more sensitive to subtle deficits, reflecting challenges in suprathreshold processing. Incorporating speech-in-noise testing alongside audiometric profiling is therefore essential for both accurate diagnosis and tailored rehabilitation. Together, these age- and diagnosis-specific patterns provide insight into the disease course and may inform more targeted rehabilitation strategies.

Swab-Based Genetic Analysis of HPV for Precise Detection and Tumor Profiling in Head and Neck Cancer

9:30 a.m. - 9:45 a.m.

Ella Jackert*¹, Swar Vimawala², Liyang Tang², Daniel Kwon², Niels Kokot², Uttam Sinha², Albert Han²

¹*Keck School of Medicine of USC*, ²*USC Caruso*

Background: Current methods to detect and genotype human papillomavirus (HPV) in oropharyngeal squamous cell carcinoma (OPSCC) rely on tissue specimens or assays with limited specificity. We validated a minimally invasive swab-based strategy with potential to improve diagnosis, tumor characterization, and surveillance.

Methods: Twenty-five OPSCC and 20 oral cavity cancer patients were included. All controls tested HPV-negative. HPV16 was detected in 21/25 OPSCC tumor swabs (84%). Mean HPV16 fold change was 7,503.08 in tumors versus 1.29 in contralateral normal mucosa ($p < 0.0001$). One qPCR-negative swab was HPV33-positive on sequencing. Overall sensitivity was 88% and specificity 100%.

Results: A total of 25 patients with biopsy-proven OPSCC and 20 patients with oral cavity squamous cell carcinoma were included in the study. All oral cavity samples tested negative for HPV. 21 of 25 OPSCC swab samples tested positive for HPV16 on qPCR (84%). The mean fold change of HPV16 was 7,503.08 for tumor swabs compared to 1.29 for contralateral normal oropharyngeal mucosa ($p < 0.0001$). Of the swabs that tested negative for HPV16, one swab sample was positive for MY09/MY11 and was determined to be serotype HPV33 by Sanger sequencing. The overall sensitivity was thus 88% and specificity was 100%.

Conclusions: Here, we validate a novel swab-based detection method of HPV DNA in OPSCC. This method is highly sensitive, specific, minimally invasive, and has potential for tumor characteristic including HPV genotyping that would otherwise only be able to be assessed through labor intensive tissue pathological analysis. This swab-based pathway provides a site-specific scalable diagnostic strategy that holds promise for improving early detection and strengthening surveillance of HPV-associated OPSCC in routine clinical practice.

The Molecular Landscape of Inner Ear Schwannoma

9:45 a.m. - 10:00 a.m.

Jonas Scheffler*¹, Sandra Leisz¹, Eric Lehner¹, Jessica Stieber¹, Elisa Darnstaedt¹, Sabine Koitzsch¹, Tony Gutschner¹, Arne Liebau¹, Stefan K. Plontke¹

¹*University Medicine Halle (Saale)*

Background: Inner Ear Schwannoma (IES) represent a rare subset of vestibulocochlear schwannoma (VS). Their intralabyrinthine location and close proximity to sensitive modiolar structures, including spiral ganglion and sensory hair cells, imply diagnostic, prognostic and therapeutic challenges compared to VS in the cerebellopontine angle (CPA) or internal auditory canal (IAC). Moreover, the microenvironment surrounding IES differs substantially from that of VS adjacent to the brainstem. Although clinical reports and case series on IES are steadily increasing, the tumor biology remains poorly understood. This study aims to provide a comprehensive overview of the key molecular characteristics of IES at both the RNA- and DNA-levels.

Methods: Fresh-frozen tumor samples from 15 patients with IES were collected during surgical resection. Bulk RNA sequencing was performed on all 15 samples, and whole-genome sequencing on 10. A control cohort comprised of published data of VS from CPA/IAC. Bioinformatic analyses included differential gene expression, pathway enrichment, cell-type deconvolution, as well as variant calling, copy-number/ structural variation analysis, and mutational signature profiling. Integrated RNA–DNA analyses were conducted to characterize molecular features of IES in relation to their specific location within the inner ear.

Results: Integrative genomic and transcriptomic analyses identified molecular features of IES. The datasets showed alterations at transcriptional levels, involving pathways relevant to tumor biology, location and the tumor microenvironment.

Conclusions: This study provides the first integrative molecular characterization of IES. These findings advance the understanding of IES biology and provide a foundation for the development of improved diagnostic and therapeutic approaches.

Podium 4: Multi-disciplinary Research of the Middle Ear

8:00 a.m. - 10:00 a.m.

Meeting Room 208ABC, Floor 2

Moderators: Regina Gendzelevski Kelmann and Z. Jason Qian

Impact of Age and Sensorineural Hearing Loss on Sound Transmission Through the Middle Ear

8:00 a.m. - 8:15 a.m.

Stefan Stenfelt*¹

¹*Linköping University*

Background: Sound transmission typically occurs through air conduction (AC): sound waves enter the outer ear, are converted into vibrations by the tympanic membrane, and are transmitted via the ossicles to the inner ear. Bone conduction (BC) provides an alternative pathway, bypassing the middle ear. Traditionally, middle ear sound transmission—assessed through the difference between AC and BC thresholds—has been regarded as stable and unaffected by aging or sensorineural hearing loss (SNHL). However, recent evidence suggests that age and SNHL may influence this relationship. The present study aimed to evaluate how aging and SNHL affect middle ear sound transmission, measured as the AC–BC threshold difference.

Methods: Audiometric data from 13,417 participants (ages 4–102 years, mean 59.3 years) were extracted from the Linköping University Hospital audiology database (2018–2019). Bilateral AC and BC thresholds between 250–4000 Hz were analyzed. Ears with presumed conductive hearing loss, defined as ≥ 25 dB difference between AC and BC at two or more frequencies, were excluded. Effects of age, gender, and SNHL on AC–BC differences were assessed using regression modeling.

Results: Across the cohort, significant AC–BC differences were observed: ~ 5 dB at 250, 1000, and 3000 Hz, and 11 dB at 4000 Hz. Females demonstrated slightly greater differences (~ 1 dB) than males, likely reflecting reference threshold standards. Children and adolescents (< 20 years) exhibited higher AC–BC discrepancies than adults. Regression analysis showed negligible influence of age on AC–BC differences, except at 500 Hz where middle ear transmission improved slightly (~ 0.5 dB per decade). When AC thresholds were used as reference, regression models indicated poorer middle ear transmission at ≤ 1 kHz with greater hearing loss, whereas using BC thresholds suggested the opposite—improved transmission. At higher frequencies, both reference systems indicated worsening transmission with increased loss. However, detailed analysis suggested these findings were largely attributable to measurement constraints, particularly at the extremes of AC and BC testing ranges.

Conclusions: Children exhibited systematically larger AC–BC differences, likely due to developmental or calibration-related factors, producing spurious indications of conductive loss. Small gender-related differences appear to arise from anatomical variation. In adults, middle ear transmission remains stable across age, with no meaningful impact of SNHL. Previously reported effects of aging or SNHL on middle ear sound transmission are most likely artifacts of test limitations, particularly with BC at threshold extremes. Additional contributors to variability may include vibrotactile responses, harmonic distortion, airborne radiation, binaural summation, and calibration differences between AC and BC transducers.

Improved Automatic Segmentation of the Ossicular Chain From Cone-Beam Ct Using Nnu-Net With an Expanded Dataset of 2,000 Scans

8:15 a.m. - 8:30 a.m.

Liam Barrett^{*1}, Talisa Ross², Anne Schilder³, Nishchay Mehta²

¹*University College London*, ²*Royal National Ear Nose and Throat Hospital*, ³*Ear Institute University College London*

Background: Automated segmentation of the ossicular chain from medical imaging is crucial for otological diagnosis, surgical planning, and treatment monitoring. Whilst convolutional neural networks have shown promise in middle ear segmentation, existing studies have been severely limited by dataset size. Our systematic review identified only 866 scans across all published studies, with individual studies typically using fewer than 200 scans. The stapes has proven particularly challenging, with even the best-performing architectures achieving Dice similarity coefficients of only 0.84, compared to over 0.90 for the malleus and incus. This research addresses these limitations by leveraging a substantially larger dataset to determine whether increased training data improves segmentation performance, particularly for challenging anatomical structures.

Methods: We utilised 2,000 cone-beam CT scans from University College London Hospitals, representing a three- to ten-fold increase over previous studies. Between 200 scans were manually annotated by expert clinicians for the complete ossicular chain and individual components (malleus, incus, stapes). The nnU-Net architecture was selected based on its self-configuring capabilities and proven performance in medical image segmentation. We trained three model variants: 3D low-resolution, 3D full-resolution, and 3D cascade, using five-fold cross-validation. Performance was evaluated using Dice similarity coefficient, average symmetric surface distance, modified Hausdorff distance, and 95th percentile Hausdorff distance. We investigated the relationship between training set size and segmentation accuracy through incremental training experiments.

Results: Analysis indicates improved segmentation performance across all ossicular components compared to literature benchmarks. The expanded dataset demonstrates particular benefit for stapes segmentation, historically the most challenging structure. Performance scaling analysis reveals a distinct pattern in the relationship between dataset size and segmentation accuracy across ossicular structures. The malleus and incus achieved state-of-the-art performance after approximately 70 training scans, with Dice coefficients plateauing at 0.92 and 0.93 respectively. In contrast, stapes segmentation continued to improve with increasing dataset size up to 120 scans (Dice=0.88), demonstrating that smaller anatomical structures benefit disproportionately from larger training datasets. Notably, we did not observe a ceiling effect in model capacity; more complex architectures (3D full-resolution and 3D cascade) consistently outperformed simpler models without exhibiting overfitting, suggesting that both data quantity and model complexity contribute to optimal performance. These results demonstrate that the combination of expanded training data and sophisticated architecture selection enables clinically viable automated segmentation across all ossicular components.

Conclusions: This study demonstrates that substantially larger training datasets can overcome historical limitations in automated ossicular chain segmentation, particularly benefiting small anatomical structures like the stapes. Our findings suggest that data scarcity has been a significant constraint in achieving clinically viable segmentation accuracy. These results have immediate implications for clinical workflow integration, potentially enabling automated preoperative planning and quantitative assessment of middle ear pathology. Future work should focus on validating performance across multiple institutions and extending to pathological cases.

Background and Procedure-Related Histologic Findings in Otic Tissues of Four Laboratory Species

8:30 a.m. - 8:45 a.m.

Cory Howard*¹, Rachel Tapp², Mee Ja Sula²

¹Michigan State University Veterinary Diagnostic Lab, ²Charles River Laboratories

Background: Preclinical ototoxicity studies evaluate drug candidates intended for human use and typically include histologic examination of the external, middle, and inner ear, along with auditory brainstem response (ABR) and cytochleogram assessments. Larger mammalian species such as guinea pigs, rats, nonhuman primates, and chinchillas are commonly used as animal models. Despite the importance of these studies for investigational new drug applications to proceed with clinical trials, limited information is available on background and procedure-related histologic findings in control animals. Such information is critical for determining whether observed changes represent test article-related effects.

Methods: Histologic observations were retrospectively collected from ototoxicity studies conducted at Charles River Laboratories (formerly MPI Research; Mattawan, MI). Only control groups (vehicle- or placebo-treated animals) were included. Fifty-one studies met inclusion criteria, encompassing 42 cynomolgus macaques, 437 guinea pigs, 217 Sprague Dawley rats, and 56 cats. Observations were grouped by route of administration: cynomolgus macaques received transtympanic injection through the tympanic membrane with deposition of test article into the tympanic cavity (n = 31) or mastoidotomy and direct administration into the cochlea via the round window membrane or semicircular canals (n = 11); guinea pigs received middle ear catheter (n = 105), transtympanic (n = 268), or systemic (n = 64) administration; rats received systemic (n = 83), transtympanic (n = 128), or topical (n = 6) administration; and cats received only transtympanic administration (n = 56). Histopathologic observations were combined regardless of severity or laterality. Percent incidence was calculated for each observation.

Results: Distinct species-specific patterns were observed. In systemically treated guinea pigs, the most common histologic findings were new bone formation in the ossicles (40.6%) and tympanic bulla (35.9%), while in Sprague Dawley rats, mucoperiosteal mineralization (31.3%) and tympanic cavity infiltration/inflammation (26.5%) were most common. Guinea pigs with indwelling catheters had middle ear new bone formation (7.6%), as well as extensive fibroplasia (45.7%) and inflammation (35.2%) centered on the catheter insertion site. In cynomolgus macaques, tympanic cavity new bone formation and fibroplasia were seen in all animals (100%) undergoing mastoidotomy and intracochlear administration while only a few animals (6.5%) undergoing transtympanic administration had new bone formation. Mastoidotomy was also associated with hemorrhage (45.5%), foreign material (90.9%) and inflammation (54.5%) in the tympanic cavity. Tympanic membrane fibrosis was common in both mastoidotomy (81.8%) and transtympanic (77.4%) administration. Across all species and routes of administration, pigmented/foamy macrophages in the tympanic cavity was common.

Conclusions: Species and route of administration affect the background and procedure-related incidence of histologic findings in preclinical ototoxicity studies. These observations must be considered when determining whether findings represent test article-related effects.

The Effects of Basic Fibroblast Growth Factor and Gelatin Sponge Administration on Blast-Induced Tympanic Membrane Perforation in Mice

8:45 a.m. - 9:00 a.m.

Shingo Yasutake^{*1}, Kunio Mizutani², Takaomi Kurioka³, Yasushi Satoh¹

¹National Defense Medical College, ²Tokyo Women's Medical University Adachi Medical Center, ³Kitasato University School of Medicine

Background: In recent conflicts and terrorism, blast injuries caused by bombs and explosives are a common possibility. Although tympanic membrane perforation (TMP) is known to be the most frequent auditory complication of blast injuries, its healing rate is generally poor. While basic fibroblast growth factor (bFGF)-containing gelatin sponges (gel) have been shown to be effective in the treatment of TMP, studies on their use for blast-induced TMP haven't been conducted. Using and developing a blast injury mouse model, we have previously researched inner ear and middle ear injuries. In the present study, we investigated the effects of bFGF and gelatin sponge administration on blast-induced TMP in mice.

Methods: Male CBA/J mice, 10-12 weeks of age, were divided into five groups: (1) control (non-blast-exposed), (2) no treatment (untreated blast-exposed TMP ears), (3) bFGF (blast-exposed TMP ears treated with bFGF eardrop), (4) gel (blast-exposed TMP ears treated with gel placement into the TMP), and (5) bFGF + gel (blast-exposed TMP ears treated with bFGF immersed gel placement into the TMP). The blast-tube system generated a peak pressure of 330 ± 11.3 kPa from the blast shock wave and irradiated the mice from the front of their heads. TMP size was measured over time, auditory function was assessed using auditory brainstem response (ABR) and distortion product otoacoustic emissions (DPOAEs), and cochlear damage was evaluated via hair cell survival. Two months after the blast exposure, the fixed middle ear of mice embedded in paraffin, and the tympanic membrane (TM) was histologically evaluated for thickness.

Results: Combination treatment with bFGF and gel promoted better TMP healing, with thinner, structurally improved tympanic membranes compared to other groups. There were no effects of drug administrations, such as bFGF or gel, on ABR, DPOAE and hair cell survival rates.

Conclusions: These findings suggest that bFGF with a gelatin scaffold may effectively enhance TMP repair following blast injuries. This study provides new insights into potential therapeutic strategies for blast-related auditory injuries, which we will discuss alongside detailed auditory and histological assessments.

Osteoclasts Begin to Migrate From the Perivascular Area in Lps Induced Otitis Media Mouse Model

9:00 a.m. - 9:15 a.m.

Sho Kanzaki^{*1}, Yukiko Kuroda², Koichi Matsuo²

¹National Institute of Sensory Organ Tokyo Medical Center, ²Laboratory of Cell and Tissue Biology, Keio University School of Medicine, Tokyo, Japan

Background: Recurrent or chronic otitis media has long been associated with hearing loss, yet the mechanisms that underlie this relationship remain incompletely defined. One possible explanation is that hearing loss arises as a consequence of inflammation-induced activation of osteoclasts, the specialized bone-resorbing cells. Inflammatory conditions create an acidic microenvironment that weakens the bone matrix, and osteoclasts accelerate this degradation, leading to histologically distinct resorption. Repeated or chronic episodes of otitis media are more likely to result in progressive bone destruction. However, it is not yet clear whether acute otitis media itself is sufficient to mobilize osteoclasts and initiate ossicular bone damage, or from which cellular niche these osteoclasts arise. Clarifying the dynamics of osteoclast recruitment in the setting of otitis media is therefore essential to understanding disease-related bone pathology and its link to hearing loss.

Methods: To investigate this question, we established an experimental model of otitis media in mice. Lipopolysaccharide (LPS), a component of Gram-negative bacterial cell walls known to elicit strong inflammatory responses, was injected into the middle ear cavity to induce otitis media. As a control, phosphate-buffered saline (PBS) was injected into the middle ear of separate mice. Ossicles were harvested for histological and imaging analyses. Osteoclast localization was assessed using tissue sections, and three-dimensional visualization was achieved with light-sheet microscopy on optically cleared ossicular samples. This approach allowed us to evaluate not only the number of osteoclasts but also their spatial relationship to surrounding vascular structures.

Results: Our analyses revealed the presence of osteoclasts in perivascular regions of the ossicles. A higher number of osteoclasts was observed in LPS-treated mice compared to PBS controls, with the malleus demonstrating the most pronounced difference. These findings indicate that osteoclast activity is upregulated in response to LPS-induced middle ear inflammation. Importantly, the perivascular positioning of these osteoclasts suggests that blood vessels act as a key site for their recruitment and activation,

Conclusions: Osteoclasts were consistently found in perivascular regions within the ossicles of LPS-inoculated mice. The perivascular localization of osteoclasts implies that osteoclasts may originate from circulating precursors and subsequently contribute to bone resorption from within the ossicular chain. This osteoclastic activity may create focal porosity in the ossicles during otitis media, thereby weakening their structural integrity. Our findings highlight a potential mechanism by which inflammatory processes in otitis media drive osteoclast formation and subsequent ossicular bone resorption. This insight not only advances understanding of how chronic infections lead to hearing loss but also points to possible preventive strategies that target osteoclast recruitment or function, with the ultimate goal of mitigating disease-related auditory impairment.

Regulation of Middle Ear Genes During Otitis Media by Changes in Dna Accessibility

9:15 a.m. - 9:30 a.m.

Allen F. Ryan^{*1}, Nathan Zemke¹, Arwa Kurabi¹

¹*University of California, San Diego*

Background: The middle ear (ME) mounts a three-pronged initial defense when invaded by bacteria: mucus, innate immune receptors, and effectors including cytokines, chemokines and

antimicrobial peptides which are mobilized when innate immune receptors are activated. While gene expression studies document these defense processes during otitis media (OM), how they are regulated is less well known.

Methods: Single-cell RNA-Seq was used to assess expression of genes, including regulatory transcription factors (TFs), throughout an episode of acute OM in the mouse. Single-nuclear ATAC was employed to document changes in defense gene DNA accessibility in the neighborhood of their transcribed regions. Transcription factor binding sites within altered DNA regions were mapped, and compared to TF expression.

Results: OM-associated changes in DNA accessibility were identified for many defense genes whose expression was also altered, exposing binding sites for TFs. For other defense genes, accessibility changes were minimal. For a few genes, some accessible regions were eliminated by infection. TFs associated with binding sites in accessible regions that showed significant changes during OM included HIF1A.

Conclusions: Changes in DNA accessibility appear to be involved in the regulation of many genes expressed by ME cells during otitis media. The presence of HIF1A binding sites, coupled with increased expression during OM, suggests a role for hypoxia in middle ear gene regulation. For genes in which changes in DNA accessibility were minimal or absent, altered expression may be regulated by changes in TF expression, DNA methylation, and/or alterations in DNA accessibility at distant sites.

Rare and Low-Frequency Genetic Variants in Families With Otitis Media

9:30 a.m. - 9:45 a.m.

Regie Lyn Santos-Cortez¹, Christina Elling¹, Helen Gomez¹, Elisabet Einarsdottir², Juha Kere², Petri Mattila², Lena Hafrén², Allen Ryan³

¹University of Colorado Anschutz Medical Campus, ²University of Helsinki, ³University of California San Diego

Background: Otitis media is a highly frequent diagnosis in children that causes significant morbidity but remains understudied as a genetic trait despite significant heritability in families. Our goal was to identify rare or low-frequency variants within genes that confer susceptibility to otitis media.

Methods: Exome sequence data of 287 individuals from 243 families were analyzed. Identified variants were tested for co-segregation with otitis media in family members. Genome sequence data from a case-control cohort was imputed and analyzed for association of specific genes with otitis media. Single-cell RNA-sequence data of identified genes were noted in acutely infected mouse middle ears.

Results: Thirty-three variants within 24 genes co-segregated with otitis media in 28 families, of which 18 variants were considered pathogenic or likely pathogenic. An additional 81 variants in 21 of the same genes were identified in 83 unrelated probands with otitis media. Of the 24 genes, twelve were associated with otitis media in mouse models, while fifteen genes were replicated from previous human studies. A common variant EYA4 c.829G > A was associated with otitis media in the case-control cohort. Using network analysis, twenty-two of the 24 genes were connected in a subnetwork enriched in various signaling pathways, Th1/Th2/Th17 cell

differentiation and viral infections. Majority (87.5%) of the identified genes were expressed in mouse middle ear cells, with differential expression after acute infection.

Conclusions: The identification of novel genes and variants for susceptibility to otitis media will be useful in future risk screening and clinical management in children that require a more personalized approach due to poor response to standard treatments.

Unexplained Conductive Hearing Loss in Down Syndrome: Evidence From Audiometric and Imaging Data

9:45 a.m. - 10:00 a.m.

Gabrielle Merchant^{*1}, Heather Porter¹, Kristen Janky¹, Keelin Fallon², Aaron Remenschneider³

¹Boys Town National Research Hospital, ²University of Massachusetts Chan Medical School,

³Boston Children's Hospital; Massachusetts Eye and Ear Infirmary

Background: Conductive hearing loss (CHL) affects 50-80% of individuals with Down syndrome (DS), typically attributed to chronic middle ear disease such as otitis media. However, conventional treatments like placement of pressure equalization tubes often fail to resolve CHL, and clinical observations reveal that many individuals with DS demonstrate persistent air-bone gaps without signs of active middle ear pathology. We define this audiometric pattern as unexplained conductive hearing loss (uCHL). While recognized anecdotally, uCHL remains poorly quantified and it is underrepresented in the literature. Notably, inner ear anomalies have been reported in up to 75% of individuals with DS, such as enlarged vestibular aqueducts, superior semicircular canal dehiscence, and cochlear hypoplasia. This study aims to quantify uCHL and explore its association with structural anomalies of the auditory system using audiometric and imaging data.

Methods: We analyzed two preliminary datasets:

1. Retrospective cohort: 46 ears from individuals with DS without active middle ear disease who underwent both audiologic evaluation and temporal bone computed tomography (CT) imaging within a 6-month window (Aug 2023–Aug 2024). CT scans were reviewed for anomalies of the outer, middle, and inner ear.
2. Prospective cohort: 92 ears from 46 individuals with DS, recruited regardless of hearing status, who completed behavioral audiometry, bilateral masked bone conduction testing, tympanometry, and wideband tympanometry (WBT).

Results: In the retrospective cohort, all 46 ears exhibited air-bone gaps (29 ears had CHL and 17 had mixed hearing loss), yet 70% had normal tympanometry, consistent with uCHL. CT findings showed inner ear anomalies in 91% of ears, middle ear anomalies in 83%, and outer ear anomalies in 70%, challenging the assumption that CHL in DS arises solely from middle ear dysfunction. Additional associations between imaging and audiometric data will be presented.

In the prospective cohort, 63% of ears (58/92) demonstrated air-bone gaps, and 76% of those (44/58) had normal tympanometry. WBT confirmed normal middle ear absorbance in 64% (37/58) using conservative normative criteria. Notably, masked bone conduction thresholds were frequently seen in the negative dB HL range in conjunction with air-bone gaps, suggesting

hypersensitive bone conduction. These findings were absent in neurotypical controls using identical equipment and protocols, ruling out calibration artifacts.

Conclusions: uCHL is prevalent in DS and often co-occurs with normal middle ear function on standard assessments, indicating that conventional diagnostic models may be insufficient. The high prevalence of inner ear anomalies and enhanced bone conduction thresholds suggests multifactorial or novel mechanisms, such as third window effects or altered bone conduction dynamics. These findings highlight the need for expanded diagnostic approaches in DS and further research into the pathophysiology and mechanism of uCHL in this population.

Symposium 1: In Vitro Models as Relevant Tools to Support Translational Hearing Research

8:00 a.m. - 10:00 a.m.

Meeting Room 209ABC, Floor 2

In Vitro Models as Relevant Tools to Support Translational Hearing Research

Natalia Smith-Cortinez, *University Medical Center Utrecht*

Symposium Description: Despite decades of dedicated research, hearing loss remains without a single FDA-approved therapeutic for its treatment—a stark reflection of the translational challenges facing the field. A key barrier to progress is the limited predictive power of traditional animal models and the scarcity of physiologically relevant in vitro systems that can bridge the gap between discovery and clinical application. This symposium will spotlight the need for a critical shift in regulatory and scientific strategy. We will highlight emerging high-throughput platforms and human centered strategies designed to accelerate therapeutic discovery. This include cochlear and inner ear organoids derived from patient-specific induced pluripotent stem cells (iPSCs), which offer new opportunities to model genetic forms of deafness. We will also discuss the potential of patient- or cadaver-derived cochlear stem cells for drug screening and precision medicine. This encompasses traditional models such as explants to human based organoids A particular focus will be given to recent advances in the in vitro expansion of auditory progenitor cells, which enable regenerative strategies and improve experimental scalability. By bringing together diverse voices from across auditory neuroscience, bioengineering, and the clinic, the symposium aims to catalyze interdisciplinary dialogue, inspire innovative collaborations, and build new bridges within the hearing research community. From a translational perspective, we will examine how these in vitro approaches can complement and enhance existing clinical standards, including cochlear implants, and how researchers might improve their efficacy and integration. Together, these platforms form a comprehensive translational framework for inner ear research, bridging basic science and clinical application and driving the development of next-generation therapeutics for hearing loss.

Erik de Vrieze, *Dept. of Otorhinolaryngology, Radboud University Medical Center, Nijmegen, The Netherlands*

Monica Serban, *University of Montana*

Francis Rousset, *University of Geneva*

Human Cellular Models of the Inner Ear

Konstantina Stankovic, *Stanford School of Medicine*

Individual Abstract: Hearing loss represents a significant global health challenge, reaching pandemic levels. The most prevalent form of this condition is sensorineural hearing loss (SNHL), which typically arises from damage to the inner ear's cellular structures. Human induced pluripotent stem cells (hiPSCs) can be derived from individuals, including those affected by various types of SNHL. Advancements in differentiation protocols aimed at producing inner ear cells, such as hair cells (HCs) and spiral ganglion neurons (SGNs), hold great promise for enhancing and understanding of human ear disease, evaluating potential treatments, and developing cell-based therapies. We demonstrate how hiPSCs-derived otic progenitor cells, Schwann cells, and organoids offer fresh perspective on virally-induced SNHL, including those lined to cytomegalovirus, Zika virus, and SARS-CoV-2. Additionally, we highlight how these innovative cellular models of the inner ear facilitate research into the impact of oxidative stress on the structure and function of inner ear tissues.

Human Inner Ear Organoids as a Versatile Platform to Model and Treat Acquired and Genetic Hearing Loss

Heiko Locher, *Leiden University Medical Center*

Individual Abstract: Human inner ear organoids derived from induced pluripotent stem cells (hiPSCs) are emerging as a powerful in vitro platform to study sensorineural hearing loss. These 3D models recapitulate key aspects of human inner ear development and cellular complexity, enabling investigation of both genetic and acquired causes of hearing loss. We employed inner ear organoids to model monogenic disorders, assess antisense oligonucleotide (ASO) therapies, and to study environmental insults such as ototoxic drug exposure and congenital cytomegalovirus (CMV) infection. To include relevant genotypes, inner ear organoids were generated from healthy, patient-derived, and isogenic mutant hiPSC lines. For genetic modeling, we focused on mutations in USH2A and COCH, applying ASOs via gymnotic delivery to modulate RNA expression. Organoids from healthy hiPSC lines were also exposed to known ototoxic agents (cisplatin, gentamicin) and CMV laboratory and clinical strains to assess damage and cell-type-specific vulnerability. Disease and treatment effects were evaluated using immunohistochemistry, RNAscope, qPCR, and confocal microscopy. All organoids exhibited hallmark features of the inner ear, including stereociliated hair cells and periotic mesenchyme. Patient-specific and gene-edited lines recapitulated expression of mutant transcripts and disease phenotypes. ASO treatment led to significant reductions in mutant RNA levels and partial rescue of molecular defects. Additionally, ototoxic agents and CMV infection induced selective damage to hair cells and neuronal structures, demonstrating the model's utility for studying acquired hearing loss. Our work highlights the value of using human inner ear organoids as a translational platform to dissect the molecular mechanisms underlying genetic and acquired forms of hearing loss. They enable therapeutic testing across a range of etiologies, from RNA-targeted therapies to

infection and drug-induced damage, paving the way for more personalized and predictive treatment strategies.

Progenitor Cells in the Adult Human Express Lgr5 and Have the Potential to Produce MYO7A+ Cells in Vitro

Louise Straatman, *University Medical Center Utrecht*

Individual Abstract: In the embryonic cochlea, supporting cells give rise to sensory hair cells. In adult mice, supporting cells retain expression of progenitor markers such as Lgr5 and Sox2, yet spontaneous hair cell regeneration does not occur. Most previous studies have focused on neonatal mouse cochlear explants, which limits translational relevance due to species and developmental differences, as hearing loss primarily affects adults. For translational purposes this study assessed the regenerative potential of the adult human inner ear.

We evaluated LGR5 expression in adult human inner ear tissue obtained within 12 hours postmortem from bodies donated to science. We also assessed the progenitor potential of inner ear sensory epithelium from adult patients undergoing skull-base surgery, and from young adult and aged Lgr5-GFP mice with normal hearing or hearing loss. Cells were cultured in 3D and key signaling pathways were manipulated to induce proliferation and differentiation of the cells.

Our findings show that LGR5 is expressed in the adult human cochlea and vestibular organ. Supporting cells from sensory epithelium from human inner ear and adult mouse cochlea demonstrated the capacity to proliferate and form organoids in vitro. After differentiation, MYO7A+

hair cell-like cells were generated in these organoids.

These results indicate that progenitor cells with regenerative potential are present in the human and mouse adult cochlea and vestibular organ. When activated through targeted manipulation of signaling pathways, these cells can proliferate and give rise to hair cell-like cells. This highlights their promise as therapeutic target cells for future therapies to restore hearing in adults.

A Scalable in Vitro Platform for High-Throughput Drug Development in Primary Auditory Neurons

Francis Rousset, *University of Geneva*

Individual Abstract: Sensorineural hearing loss remains an incurable condition, primarily due to

the irreversible loss of cochlear hair cells and auditory neurons. A major barrier to therapeutic development is the lack of in vitro models that faithfully recapitulate auditory neurobiology while

supporting high-throughput screening (HTS). Although rodent explants and in vivo models have provided valuable insights, their limited throughput and logistical constraints limit their utility in early-stage drug discovery, where screening large compound libraries is essential.

We have recently established a robust in vitro protocol to expand auditory neuroprogenitor cells

(ANPGs), enabling the scalable production of auditory neuron-like cells. By simultaneously activating WNT signaling and inhibiting the TGF β /Smad pathway, quiescent ANPGs are reprogrammed into a highly proliferative state. These so-called Phoenix cells combine sustained self-renewal with robust neuronal differentiation capacity, offering a stable, primary-like model system. Furthermore, they can be frozen and thawed, eliminating the need for continuous animal tissue supply while ensuring reproducible, batch-consistent assays.

Building on this foundation, we developed a high-throughput platform to screen large libraries of compounds that promote auditory neuron protection or regeneration. To enable real-time monitoring of auditory neurogenesis and neuroprotection, we implemented a live-cell genetic reporter system in 384-well plate assays. This automated system supports both phenotypic and genetic screening approaches, facilitating the discovery of novel genetic targets and candidate compounds.

Altogether, the Phoenix platform offers a scalable, reproducible, and physiologically relevant alternative to animal-based models, accelerating early-stage drug discovery and advancing the preclinical pipeline for inner ear therapeutics.

Building Your Own Toolbox: A Journey Developing and Utilizing in Vitro 3D Tissue Models to Enable High-Throughput Screening of Novel Othotherapeutics and Drug Delivery Devices

Joachim Veit, *University of Montana, Missoula*

Individual Abstract: Despite otic disorders affecting a substantial portion of the global population, othotherapeutics, particularly those intended to treat hearing loss, have seen limited development or innovation. This is in part due to the limited tool set available to the field which consequently relies heavily on costly and low throughput preclinical animal models with often ambiguous human relevance. This presentation highlights our journey in developing in vitro tympanic and round window membrane 3D human tissue models to enable high-throughput screening of topically applied othotherapeutics. These models were subsequently employed in the characterization of several novel topical therapeutics targeting the oxidation- and inflammation-mediated damage to inner ear cells which is concurrent in nearly all insults causing hearing loss. Finally, these in vitro models were utilized to prototype a series of microneedle-based drug delivery devices with tailorable tympanic residence times. These devices facilitate the transtympanic permeation of topically applied othotherapeutics to allow for recurring therapeutic access to the middle and inner ear. Together, this project highlights the value of targeted efforts to develop and make use of novel in vitro research tools which can provide lower cost, higher throughput, and increased human relevance options over preclinical animal models for translational otic research and beyond.

Cochlear Explant as a Reference Ex Vivo Model and a Platform for Developing in Vitro Systems

Marianne Aincy, *Cilcare*

Individual Abstract: Cochlear explant cultures offer a valuable and reliable approach for studying drug-induced ototoxicity and potential otoprotective effects, although validation through in vivo models remains necessary. This model provides a versatile, ethical framework suitable for multiple applications: dose-response assessments of ototoxic compounds, exploration of their mechanisms of action, and efficacy evaluation of protective treatments. It also serves as reference for developing human in vitro models such as organoids derived from hiPSCs.

To facilitate screening for otoprotective drugs while adhering to 3Rs principles (Replacement, Reduction, Refinement), we developed and optimized a robust ex vivo cochlear explant model. This model employs cochlear explants exposed to cisplatin and aminoglycosides, widely used chemotherapeutic and antibiotic agents known to cause irreversible cochlear hair cell damage and permanent hearing loss.

Our model uses neonatal rodent pups, significantly reducing animal usage by minimizing gestating females required. It offers advantages, including avoidance of systemic administration and associated ethical issues. It provides precise control over experimental conditions, ensuring consistent, direct exposure of cochlear cells to dosed treatments. The model is efficient, with standardized culture limited to 96 hours.

However, technical challenges exist. Successful explant preparation and maintenance require specialized expertise in dissection, culture media selection, and aseptic techniques. The limited lifespan of explants restricts their use in long-term studies. Additionally, the developmental immaturity of cochleae from pups aged P1-P4 may limit translational applicability to mature auditory systems. Importantly, explant models complement but do not fully replicate systemic complexity found in living organisms.

Our goal was to refine this model by addressing critical technical parameters: choice of rodent species (mouse vs. rat), pup age, dissection techniques, culture medium composition, and exposure specifics for ototoxic agents (cisplatin, kanamycin, gentamicin, tobramycin). Ototoxic severity can be adjusted by modifying these parameters to selectively target particular cochlear structures.

We validated multiple cellular markers to visualize and quantify essential cochlear components: hair cells, supporting cells, nerve fibers, ribbon synapses, and neurons. Advanced laser scanning confocal microscopy and histological analyses were employed for comprehensive qualitative and quantitative assessments.

Our cochlear explant model provides a powerful, flexible method for studying ototoxicity and identifying promising otoprotective agents, ultimately reducing reliance on in vivo testing. Building on this robust reference system, our ongoing efforts focus on developing complementary human in vitro models, including inner ear organoids and 3D-printed round window platform. Together, these models bridge fundamental research and translational applications, paving the way for innovative therapeutic approaches to hearing preservation.

Why Don't Cochlear Implants Work Better?

Manohar Bance, *University of Cambridge*

Individual Abstract: The cochlear implant-neural interface is difficult to interrogate in living CI users, and in animals the interface can differ significantly because of the size and shape of the cochlea, and different implants. CI users present with varying symptoms and complaints, and to back project to the underlying cause requires some methods to understand what is happening at the CI-neural interface. We have used a variety of models to try to understand how changes at this interface might manifest in terms of hearing performance, cross stimulation of other structures, or insertion trauma. The types of models I will discuss are: 1) Computational models which try to understand how nerves are activated by CIs, and the physical spread of current in the cochlea. 2) Physical 3D printed which help us understand insertion trauma forces, and electric field distribution inside the cochlea. 3) Cadaveric models which allow us to make measurements in realistic human like geometries. 4) Cellular models which let us measure responses of neurones to different types of stimulation, and also to simulate scarring and fibrosis. I will discuss the lessons learned from these various models, and the clinical applications of what we have learnt.

Podium 5: Auditory Prostheses #1

10:30 a.m. - 12:30 p.m.

Ballroom B, Floor 3

Cortical Encodings of Acoustic and Electrical Cochlear Stimulation Are Distinct

10:30 a.m. - 10:45 a.m.

Ariel Hight^{*1}, Michele Insanally², Julia K. Scarpa³, Yew Song Cheng⁴, Michael Trumpis⁵, Jonathan Viventi⁵, Mario Svirsky¹, Robert Froemke¹

¹New York University Grossman School of Medicine, ²University of Pittsburgh School of Medicine, ³Weil Cornell New York Presbyterian Hospital, ⁴UCSF, ⁵Duke University School of Medicine

Background: Cochlear implants are neuroprosthetic devices that restore hearing to folks with severe to profound hearing loss, providing abilities to attain open-set speech perception without aids of other sensory cues such as lip reading. Despite widespread adoption, there is limited understanding of how these devices restore functionality and the sense of hearing. One long-standing question has been how central auditory pathways are activated by stimulation by cochlear implants and to which degree this activation overlaps with activation evoked by similar acoustic stimuli under normal hearing conditions. In this study, we determined the spatial and temporal representations of both tones and cochlear implant channels across the broad extent of the auditory cortex (A1) by using micro-electrocorticography (μ ECoG), a subtype of intracranial electroencephalography (iEEG).

Methods: Stimulus evoked responses were acquired from 60 cortical surface electrodes (3.25 x 3.25 mm grid) placed over the auditory cortex. Evoked responses were analyzed using either event related potentials (ERPs, 2-100 Hz) or high gamma (HG, 70-140 Hz) signals. Tone evoked

responses (1.4-32 kHz) were measured and analyzed from N=7 normal hearing (NH) rats and cochlear-implant evoked responses were recorded and analyzed from N=7 bilaterally deafened rats unilaterally implanted with an 8-channel intracochlear array (CI). Of these rats, 4 underwent both tone-evoked and implant-evoked recordings. We trained classifiers to determine whether we could successfully decode stimulus identity from single trial iEEG measurements for both NH and CI rats-- first we reduced evoked responses using either principal component analysis (PCA) or tensor component analysis (TCA), then trained a linear discriminant analysis (LDA) decoder on 13 randomized trials and then computed prediction probabilities for the remaining trials.

Results: Both tones (NH) and intracochlear electrodes (CI) evoked clear single-trial and trial-averaged transients, and we found that A1 encoding of these responses were topographically organized for both NH and CI stimuli. Next, we found that single-trial evoked responses were more variable for CI vs NH rats, but only in the spatial and not the temporal domain. Then, we trained and tested classifiers on single trials of NH and CI evoked responses and found that stimulus identity could be correctly predicted for both NH and CI evoked responses. Lastly, for the N=4 animals with both NH and CI recordings, we trained a classifier on tone-evoked responses, tested these classifiers on intracochlear-evoked responses, and found minimal information transfer between responses evoked by both modalities.

Conclusions: Our findings provide evidence that acoustic and electrical cochlear implant stimulation evoke fundamentally distinct patterns of cortical activity. The lack of transferable representations suggest that cochlear implant users may experience qualitatively different precepts that cannot be entirely predicted by acoustic hearing. Our results also suggest that refinement of cortical responses may improve inter-neuronal variance at the population level and improve auditory perception.

Why Ramped Pulses Are Reliable Solutions for Coding Sound Intensity in Cochlear Implants : An Electrophysiological Study in the Guinea Pig Auditory Cortex

10:45 a.m. - 11:00 a.m.

Elie Partouche¹, Victor Adenis^{*2}, Pierre Stahl³, Chloe Huetz⁴, Jean-Marc Edeline⁴

¹Paris-Saclay Institut of Neurosciences, ²Mass Eye and Ear, Harvard Medical School, ³Oticon Medical/ Neurelec, ⁴NeuroPSI UMR CNRS 9197

Background: For decades, cochlear implant (CI) is the most successful neuro-prosthetic device allowing more than a million of patients to recover hearing sensation and speech understanding. The performance is usually good in silence, but CI patients have difficulties in the presence of background noise. Potentially, these limitations stem from the large spread of currents diffusing in the cochlea's perilymph when the different electrodes are activated at high current levels. To reduce the spread of current, one potential strategy is to change the shape of the electrical pulses. In human, studies have tested asymmetric rectangular pulses (Macherey et al., 2006, 2008). A new pulse shape, called "ramped pulse", has been proposed (Ballesterio et al., 2015) but only two studies have showed that such pulses elicited responses with lower thresholds than rectangular pulses (Navntoft et al 2020, Partouche et al 2023). Here, we report the consequences of using different types of ramped pulses on responses of auditory cortex (ACx) neurons to increasing electrical charges in anesthetized guinea pigs.

Methods: The intracochlear stimulating array was a shortened version of the EVO electrode array used by Oticon Medical and was composed of 6 ring-shaped Platinum-Iridium electrodes with a 0.0046mm² surface. Four shapes of ramped pulses were tested, each at 20 charge levels (from 3 to 31.5nC), to obtain the growth functions of ACx neurons. Recordings in ACx were collected by 16-channels electrodes composed of two rows of 8 electrodes. Two types of doubles ramped pulses were tested: one with the ramps on the 1st and 4th phases, a second with the ramps on the 1st and 3rd phases. We also tested single ramp pulses with the ramp on the 3rd phase

Results: Compared with the responses obtained with rectangular pulses, all ramped pulses generated (i) responses with lower thresholds (ii) higher maximal firing rates (FR) (iii) higher FR at threshold (iv) longer response latencies and (v) longer durations. These effects were always significant with cathodic-first phase, and were in the same direction (but less often significant) with anodic-first phase. The responses to increasing levels of charges were also compared with the responses to pure tones presented at eight intensities (from 5 to 75dB SPL).

Conclusions: These results indicate that ramped pulses should be considered as a good alternative to rectangular pulses, even if polarity of the first phase matters to benefit from this shape.

Comparison of Two Subjective Tests for Cochlear Implant Users Using Reverberant Speech Processed With an Established Dereverberation Algorithm

11:00 a.m. - 11:15 a.m.

Shaliza Merchant^{*1}, Christiaan Stronks¹, Nienke Langerak¹, Daphne Geijssen¹, Esther Van Marrewijk¹, Jeroen Briaire¹, Johan Frijns¹

¹*Leiden University Medical Center*

Background: Cochlear implants (CI) effectively restore speech perception in quiet conditions for individuals with severe-to-profound sensorineural hearing loss. However, speech intelligibility (SI) in background noise is severely impaired and reverberant environments are challenging for CI users. To mitigate the impact of background noise and reverberation, a variety of speech enhancement strategies have been developed. Performance is often assessed in terms of their effects on SI. However, it is known that algorithms not affecting SI may still be perceived as helpful, while algorithms that effectively enhance SI may be disliked because of speech distortion, underscoring the need for subjective assessment. Here, we compare two subjective tests using reverberant speech processed with an established dereverberation algorithm. The first test was the gold standard in subjectively perceived sound quality: the Multiple Stimulus with Hidden Reference and Anchor (MuSHRA) method. This method was compared with a simplified version, dubbed ‘MuSHRA-lite’.

Methods: Two cohorts of eighteen post-lingually deafened CI users participated. One cohort performed the MuSHRA, in which processed speech stimuli were subjectively compared to one common reference. Participants rated each stimulus on a scale from 0 - 100, for three outcome measures: SI, naturalness and listening effort, relative to an openly labeled reference of unprocessed speech and a hidden anchor. The other cohort performed the MuSHRA-lite, which

consisted of pairwise comparisons of processed speech producing preference scores for the same outcome measures. RM-ANOVA and linear mixed modeling were used to test for statistical significance between the ratings of clean speech, and reverberant and de-reverberated speech.

Results: Both methods confirmed that reverberation significantly degraded SI, naturalness, and listening effort relative to clean speech. However, the perceived benefits of the algorithm differed substantially between methods. MuSHRA-lite consistently revealed statistically significant benefits of the algorithm on all outcomes measures. By contrast, MuSHRA failed to reveal significant effects of the algorithm on any of the outcomes. This lack of statistical power could be coupled to greater variance in the data. Qualitative feedback indicated that performing the MuSHRA was experienced as challenging for many participants, the MuSHRA rating scales were also described as being “difficult” and the task overall as “confusing” (n=4/18).

Conclusions: Both methods captured the detrimental impact of reverberation, but only MuSHRA-lite revealed statistically significant benefits of the dereverberation algorithm on perceived SI, naturalness, and listening effort. Furthermore, MuSHRA-lite was less complicated to implement, easier to execute and interpret. MuSHRA-lite reflects preference between stimuli, while MuSHRA yields quality ratings, which may explain why MuSHRA results were more conservative. We conclude that MuSHRA-lite offers a practical and robust alternative for assessing subjectively perceived speech quality for CI users, especially when preference-based judgements are sufficient and participant burden must be minimized without sacrificing reliability.

Strength–Duration Analysis of Optical and Electrical Auditory Nerve Stimulation Across Species

11:15 a.m. - 11:30 a.m.

Yinchou Zhou¹, Jennifer Silva-Nash¹, Joaquin Cury¹, Xiaodong Tan¹, Claus-Peter Richter¹

¹*Feinberg School of Medicine, Northwestern University*

Background: Cochlear implants (CIs) restore hearing through electrical stimulation of spiral ganglion neurons, yet performance remains constrained by limited frequency resolution and spatial selectivity due to current spread. Optical approaches, including optogenetics (OG) and infrared neural stimulation (INS), promise greater spatial selectivity but require higher energy. While thresholds for these modalities have been reported, a systematic comparison using classic excitability metrics—chronaxie and rheobase—has not been established. Such analyses provide a unified framework for evaluating efficiency, safety, and translational potential across modalities and species.

Methods: We recorded auditory brainstem responses (ABRs) in transgenic mice carrying the optogenetic tool ChR2. We applied light at 460 nm (OG) and at 1375–1550 nm (INS) with a 400 μ m optical fibers at the round window. Electrical stimulation was performed through round window electrodes. For each modality, pulse duration (10–1000 μ s) and radiant exposure/current were systematically varied. Strength–duration curves were fit with the Weiss–Lapicque equation to extract chronaxie and rheobase.

Results: Electrical stimulation showed short chronaxies (~100–200 μ s) with rheobase energies in the sub- μ J range (0.01–0.05 μ J/pulse), confirming efficiency. OG stimulation in mice

exhibited intermediate chronaxies (~300–400 μ s) and rheobase values of ~4–6 μ J/pulse, reflecting selective but energy-intensive activation. INS required the highest energies (10–15 μ J/pulse) with chronaxies > 500 μ s. Across modalities, extending pulse duration beyond the chronaxie failed to further reduce threshold, leading to unnecessary energy expenditure.

Conclusions: By placing electrical, OG, and INS stimulation into a single strength–duration framework, this study identifies fundamental efficiency gaps and defines the optimal temporal regimes for each modality. Cross-species analysis highlights how cochlear anatomy shapes chronaxie/rheobase, underscoring the translational value of guinea pig models. Importantly, the findings support hybrid coding strategies: electrical pulses delivered at threshold can prime neurons, thereby reducing optical thresholds and minimizing thermal load while preserving spatial selectivity. This work provides a physiological basis for designing energy-efficient, opto-electrical cochlear implants aimed at improving pitch perception and spectral resolution in human users.

Effect of Electrode Array Type and Surgical Approach on Structure Preservation Rates During Cochlear Implantation: A Prospective Randomized Controlled Trial (CIPRES Study)

11:30 a.m. - 11:45 a.m.

Imogen van Beurden^{*1}, Saad Jwair¹, Robert J. Stokroos¹, Huib Versnel¹, Hans G.X.M. Thomeer¹

¹*University Medical Center Utrecht*

Background: Preservation of residual low-frequency hearing during and after cochlear implantation contributes to improving hearing with a cochlear implant (CI), especially in noisy environments. Scalar translocation (STL) is a major threat to hearing preservation during cochlear implantation surgery. Selection of the optimal combination of electrode array and surgical approach could add to better preservation of residual hearing and thus improved hearing with a CI.

Methods: This study is still ongoing. The last participant will be included in September 2025. Forty-seven of 48 adult patients with severe to profound sensorineural hearing loss (SNHL) receiving a CI in a tertiary referral center (UMC Utrecht) were included between January 2020 and September 2025 in an ongoing prospective randomized controlled trial (RCT) comparing surgical approaches (round window or cochleostomy insertion) and electrode arrays models (lateral wall SlimJ array (LW) or perimodiolar MidScala (PM) array, by Advanced Bionics). Participants were randomly assigned to one of four study groups, with 12 participants each: group 1 RW + LW array; group 2 CO + LW; group 3 RW + PM array (Jwair et al., 2021, *Trials* 22:895). Participants underwent preoperative high resolution computed tomography (HRCT) of the mastoid. Postoperative cone beam computed tomography (CBCT) imaging was performed within five hours after surgery. A radiological tool as developed by Advanced Bionics was used to analyze implant array position by pre- and postoperative imaging in terms of occurrence of STL and insertion depth (aDOI). STL rates and aDOI were compared between all groups.

Results: Analyses pre- and postoperative imaging of 43 participants have been performed thus far, of which 34 (9/11 in group 1; 7/10 in group 2; 8/10 in group 3; 10/12 in group 4) were

successful. Preliminary results showed that STL was observed in 9/34 (26.5%) successfully analyzed images. For group 1 (RW + LW) STL occurred in 1/9 participants (11.1%). For group 2 (CO + LW) STL occurred in 2/7 participants (28.6%). For group 3 (RW + PM) STL occurred in 3/8 participants (37.5%). For group 4 (CO + PM) STL occurred in 3/10 participants (30%). A mean cochlear width B of 6.93 (± 0.35) mm was found in the first 43 participants. A mean aDOI of 405 (± 45) degrees was found in the 34 successfully analyzed images. After completion of all 48 inclusions, between group analysis will be conducted of pre- and postoperative images to compare results between the four study groups.

Conclusions: To improve hearing preservation, preoperative planning before cochlear implantation surgery could potentially include the selection of an electrode model in combination with a surgical approach.

Post-Surgical Hearing Loss With Cochlear Implantation: Measures and Models

11:45 a.m. - 12:00 p.m.

Rachel Scheperle¹, Stephen Neely², Peizhe Wu¹, Marlan Hansen¹

¹*University of Iowa Hospitals and Clinics*, ²*Boys Town National Research Hospital*

Background: Preservation of acoustic hearing post cochlear implantation (CI) is achievable, though with variable success rates (Snels et al., 2018; Gantz et al., 2022). Post-surgical changes in hearing status can be categorized according to their underlying mechanism (e.g. middle ear sound transmission, cochlear transduction, or neural excitation). Identifying the responsible mechanism is critical to hearing preservation and influences audiological management. This project aims to evaluate the changes observed in longitudinal electrocochleography (ECochG) measurements in relation to behavioral thresholds, and to assess whether a cochlear model can provide insights regarding the identification of underlying mechanisms.

Methods: Forty-eight CI recipients with varying degrees of residual acoustic hearing are enrolled in a longitudinal study that includes serial measurements of behavioral thresholds and ECochG. ECochG were elicited at a supra-threshold level using alternating-phase tonebursts. The difference waveform was Fourier transformed. Both magnitude and phase at the stimulus frequency were tracked longitudinally to compare with behavioral thresholds. Exemplar data sets were chosen to compare with the cochlear model, with attention to changes in measurements elicited at 500-Hz.

A time-domain model of cochlear mechanics that includes a middle-ear preprocessing stage followed by a cochlear processing stage was used to produce a simulated ECochG (Neely and Rasetshwane, 2017). The model was extended to estimate the voltage at an intracochlear location, which was adjusted to fit individual ears, by including volume-conduction impedance to combine individual OHC current across the cochlea (van Gendt, 2020). Selected model parameters were then manipulated to produce a combination of middle ear conductive hearing loss (CHL) and outer hair cell (OHC) dysfunction that would simulate the 500-Hz threshold shift observed in the exemplar ears. The effects of these model manipulations on delay of the simulated ECochG were compared to the measured ECochG.

Results: Behavioral hearing thresholds are typically poorest in the first weeks after implantation and recover slightly across subsequent weeks or months. While ECoChG amplitude reductions follow roughly similar patterns as threshold shifts, delay changes could be either positive or negative. Threshold shifts and ECoChG delay changes were simulated by a specific combination of CHL and OHC dysfunction. The phase of middle-ear input impedance in the model was affected by CHL but not OHC dysfunction.

Conclusions: Changes in acoustic hearing status post CI reflect various underlying mechanisms. Although (partial) recovery of audiometric thresholds at early time points has been interpreted to suggest a transient conductive loss, comparison of ECoChG delay with model results suggest intracochlear contributions may also be present. In future work, middle-ear input impedance estimated from acoustic measurements could have value in distinguishing whether threshold losses are conductive or sensory.

Otogenetic Stimulation in a High-Resolution Model of Human Cochlea

12:00 p.m. - 12:15 p.m.

Siwei Bai¹, Albert Croner¹, Guanyu Chen¹, Lukas Driendl¹, Werner Hemmert*¹

¹*Technical University of Munich*

Background: Conventional cochlear implants (CIs) are one of the most successful achievements of medical technology. However, their performance is limited by the wide spread of electric current in the highly conductive perilymph inside scala tympani. As a solution, optogenetic cochlear implants (oCIs) may achieve a much more focal stimulation. As the cochlea in humans is embedded within the skull and thus not directly accessible to electrophysiological recordings, computational models of the human cochlea are indispensable to gain more insight into how oCIs excite fibers within the population of auditory nerve fibres and how the excitation patterns compare with conventional CIs.

Methods: An anatomically realistic model of the cochlea and auditory nerve was reconstructed from a high-resolution μ CT scan of a human cochlea with 6 μ m voxel size (Bai et al., 2019). In this preliminary study, we acquired only half of the basal turn of the cochlea with the nerve. We imported the model into Ansys Speos to perform Monte-Carlo ray-tracing simulations of light spread in the cochlea. Volumetric optical properties of the model tissues were based on Khurana et al. (2022), and surface properties were based on Ugnell and Öberg (1997) and Stelzle et al. (2010, 2011). Both waveguide-based and LED-based oCIs were simulated with an emission power of 30mW and a wavelength of 655nm, facing directly towards Rosenthal's canal. The incident irradiance values were obtained along the centerline of Rosenthal's canal, along the centerline between Rosenthal's canal and the edge of the spiral lamina, and along the auditory neural fibre closest to the emitter.

Results: The number of rays from the emitter has an impact on the convergence of the simulation outcomes, and we found that five million rays provided the optimal convergence for our simulations. Using more realistic surface properties, compared to the Fresnel Laws, waveguide-based oCIs produced a slightly more diffuse emission profile, whereas LED-based oCIs produced a slightly more focal profile. Their maximum irradiances were, however, all reduced.

Conclusions: The passive waveguide oCIs resulted in a much more focal and more intense stimulation at the region where the emitter was facing; the smaller the numerical aperture was, the stronger the effect it showed. While this type of emitter has a potential benefit of supreme spectral selectivity, it may be extremely sensitive to the implantation technique, as it requires precise orientation. In comparison, the LED-based oCIs resulted in a broader stimulation, and the strongest irradiance appeared not at the soma, where the emitter was facing, but at the peripheral axon.

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Uncovering Patterns in Post-Operative Cochlear Implant Hearing Outcomes Through Group Based Trajectory Modeling

12:15 p.m. - 12:30 p.m.

Connie Chang-Chien¹, Max Jiam¹, Yew Song Cheng¹, Nicole Jiam¹

¹*University of California - San Francisco*

Background: While machine learning methods have increasingly been applied to predict cochlear implant (CI) outcomes, they primarily focus on cross-sectional predictions rather than capturing the dynamic, longitudinal rehabilitation progress. This study aims to apply GBTM (group-based trajectory modeling) to longitudinal WRS data to uncover discrete rehabilitation tracks post-implantation, addressing a critical gap in personalized outcome monitoring and intervention.

Methods: A retrospective cohort study was conducted on 122 adult CI users with bilateral sensorineural hearing loss implanted at a tertiary academic center. The primary tracked outcome was word recognition score (WRS) per appointment from pre-operative baseline to 60 months post-implantation. Patient demographic information and bilateral pre-operative PTA and WRS for both implanted and contralateral ears were included as GBTM context. Missing data were imputed using forward and backward fill methods, and trajectories were standardized by converting each time point measurement to z-scores. Longitudinal trajectory analysis was performed using Gaussian Mixture Models (GMM) to identify distinct outcome groups by minimizing entropy via Bayesian Information Criterion (BIC), first with all patient data, then for two age-stratified groups (age at implantation < 60 and ≥60). Statistical analysis of covariate group differences was evaluated based on data distributions.

Results: GBTM identified three general trajectory groups across all patients (BIC=-8524.5, $p < 0.001$), with significant separation for age at implantation (Kruskal-Wallis statistic (KW)=7.10, $p=0.029$). For the < 60 age cohort (n=31), we identified three trajectory groups (BIC=504.9, $p < 0.001$) with significant non-zero slopes, where implanted ear pre-operative WRS was the only

significant predictor of group membership (KW=6.58, $p=0.037$). The implanted ear pre-operative WRS for < 60 sub-groups were 6.56 ± 11.30 , 17.60 ± 17.5 , and 26.42 ± 21.72 , with trajectory slopes of 0.231, 0.428, and 0.149 via least squares linear regression ($p < 0.05$). In the ≥ 60 age cohort ($n=91$), we identified four trajectory groups (BIC=780.6, $p < 0.05$), but only one with a significant non-zero slope (-0.286 , $p=0.003$). Notable features for the down-trending subgroup included a lower pre-implantation WRS in the implanted ear (17.79 vs 19.56-24.24), but having a similar contralateral ear PTA and WRS, implantation age, and demographic features as others in the same age cohort.

Conclusions: Younger adults (< 60) exhibited more predictable rehabilitation trajectories characterized by pre-implantation baseline hearing status, with those starting with lower pre-operative implanted ear WRS and private insurance demonstrating the most rapid early improvements. In contrast, older patients (≥ 60) exhibited greater rehabilitation heterogeneity, including a subgroup with poor post-CI adaptation and no distinguishing study demographic and clinical predictors besides a lower implanted ear pre-operative WRS compared to other patients in the same age cohort. These findings highlight that younger adult CI users may follow more uniform recovery pathways, while older adults may require closer longitudinal monitoring and individualized interventions. Incorporating trajectory-based models into clinical care could enhance risk stratification, guide rehabilitation strategies, and inform counseling about expected recovery patterns.

Podium 6: OTOF Gene Therapy Trial Updates and New Targets: GJB2 and STRC

10:30 a.m. - 12:30 p.m.

Meeting Room 209ABC, Floor 2

Moderators: Jeffrey R. Holt and Aara Patel

International Expert Consensus on Gene Therapy for Hereditary Hearing Loss: Based on Clinical Trials

10:30 a.m. - 10:45 a.m.

Xiaoyun Chen^{*1}, Xintai Fan¹, Lukas Landegger², Tobias Moser³, Fan-Gang Zeng⁴, Robert Nash⁵, Wade Chien⁶, Dan Jiang⁷, John Greinwald⁸, Manohar Bance⁹, Manuel Manrique Rodríguez¹⁰, Sang-Yeon Lee¹¹, Chen-Chi Wu¹², Barbara Vona¹³, Nicola Strenzke¹³, Dirk Beutner¹³, Nikul Amin⁵, James Arwyn-Jones¹⁴, Deepak Chandrasekharan⁵, Yen-Fu Cheng¹⁵, Xue Zhong Liu¹⁶, Shiming Yang¹⁷, Hao Wu¹⁸, Zhengmin Wang¹⁹, Huawei Li¹⁹, Jay Rubinstein²⁰, Lawrence Lustig²¹, Renjie Chai²², Zheng-Yi Chen²³, Yilai Shu¹

¹ENT Institute and Department of Otorhinolaryngology, Eye and ENT Hospital, Fudan University, ²Stanford University School of Medicine, ³Institute for Auditory Neurosciences and InnerEarLab, University Medical Center Göttingen, ⁴University of California Irvine, ⁵Great Ormond Street Hospital, ⁶Johns Hopkins School of Medicine, ⁷St. Thomas Hospital, Guy's and

St. Thomas' NHS Foundation Trust, ⁸Cincinnati Children's Hospital, University of Cincinnati College of Medicine, ⁹University of Cambridge, ¹⁰Clínica Universidad de Navarra, ¹¹Seoul National University Hospital and Seoul National University College of Medicine, ¹²Taiwan University Hospital, ¹³University Medical Center Göttingen, ¹⁴Great Ormond Street Institute of Child Health, ¹⁵Taipei Veterans General Hospital, ¹⁶University of Miami Miller School of Medicine, ¹⁷Chinese PLA General Hospital, Chinese PLA Medical School, ¹⁸Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, ¹⁹ENT Institute, Eye and ENT Hospital, Fudan University, ²⁰University of Washington, ²¹Columbia University Medical Center, ²²Southeast University, ²³Massachusetts Eye and Ear/Harvard Medical School

Background: Hereditary hearing loss is one of the most common disabling disorders in children and lacks effective pharmacological treatments. Recent breakthroughs in OTOF gene therapy clinical trials necessitate standardized frameworks to guide emerging therapies. This study aimed to establish the first international consensus on clinical application of gene therapy for hereditary hearing loss.

Methods: A modified Delphi process was conducted from March 2024 to March 2025, involving 46 multidisciplinary experts from several countries across otology, genetics, audiology, gene therapy, and hearing rehabilitation. After a systematic literature review, as well as integration of research and clinical expertise and experience, three iterative voting rounds (two anonymous surveys and one online consensus meeting) were performed. Statements required $\geq 75\%$ agreement for inclusion.

Results: From 9093 publications, 69 were used to draft and support the consensus statements. A total of 30 statements relevant to six domains achieved consensus on gene therapy for hereditary hearing loss, including ethical review (1 statement), patient selection criteria (12 statements), diagnosis and preoperative evaluation (9 statements), gene therapy drug delivery (4 statements), follow-up (3 statements), and post-treatment auditory and speech rehabilitation (1 statement).

Conclusions: This consensus provides the first globally endorsed framework for gene therapy in hereditary hearing loss. It standardizes clinical trial design and patient management, accelerating translation from research to practice while ensuring safety. The guidelines are immediately applicable to OTOF-related hearing loss and adaptable to other genetic forms.

Multicenter Gene Therapy for Otof-Related Deafness Followed up to 2 Years

10:45 a.m. - 11:00 a.m.

Yilai Shu^{*1}, Luoying Jiang¹, Xiaoting Cheng¹, Jun Lv¹, Yuxin Chen¹, Xiaoyun Chen¹, Liqin Zhang², Lei Han¹, Xin Zhang¹, Qi Cao¹, Daqi Wang¹, Wei Yuan³, Qin Wang⁴, Guodong Feng², Huawei Li¹, Zheng-Yi Chen⁵

¹ENT Institute, Eye and ENT Hospital, NHC Key Laboratory of Hearing Medicine, Institutes of Biomedical Sciences, State Key Laboratory of Medical Neurobiology and MOE Frontiers Center for Brain Science, Shanghai Key Laboratory of Gene Editing and Cell Therapy for Rare Diseases, Fudan University, ²Peking Union Medical College Hospital³Chongqing General

Hospital (The Affiliated Hospital of Chongqing University) ⁴The Second Xiangya Hospital, ⁵Harvard Medical School

Background: Autosomal recessive deafness 9 (DFNB9), caused by OTOF gene mutations, is characterized by prelingual or congenital, severe-to-complete bilateral hearing loss. While the benefit of gene therapy has been proven in a limited number of DFNB9 patients, its efficacy and safety across a broader age range and longer follow-up remain to be evaluated.

Methods: In this single-arm, multicenter trial conducted at 4 centers, patients aged ≥ 6 months with severe-to-complete hearing loss resulting from OTOF mutations were eligible. Patients receive a single cochlear administration of AAV1-hOTOF via the round window membrane at three vector dose groups. The primary endpoint was dose-limiting toxicity within 6 weeks. The secondary endpoint assessed efficacy, including auditory function and speech perception, and safety including adverse events.

Results: 31 DFNB9 participants (aged 1.0 to 32.3 years) received gene therapy, with a follow-up up to 2 years. No dose-limiting toxicities or serious adverse events were observed. Grade 3 events included decreased neutrophil count (3 of 31). Hearing recovered in 90% (28 of 31) of participants, with the improvement of the auditory brainstem response thresholds from > 96 dB at baseline to 55, 51, and 37 dB at 1, 1.5, and 2 years, respectively. Participants with hearing recovery exhibited an improvement in speech perception.

Conclusions: AAV1-hOTOF is well-tolerated and efficacious across a broader patient population, with sustained therapeutic benefits up to 2 years.

Safety and Efficacy of DB-OTO Gene Therapy in Children With Profound Deafness Due to Otoferlin Variants: Data From the Chord First-In-Human Registrational Trial

11:00 a.m. - 11:15 a.m.

Eliot Shearer^{*1}, Manohar Bance², Daniela S. Carvalho³, John H. Greinwald Jr.⁴, Steven A. Harvey⁵, Akira Ishiyama⁶, Evie C. Landry⁷, Hubert Löwenheim⁸, Lawrence R. Lustig⁹, Manuel Manrique¹⁰, Robert Nash¹¹, Rubén Polo¹², Cedric V. Pritchett¹³, Jay T. Rubinstein¹⁴, Ignacio del Castillo¹⁵, Jeffery J. Anderson¹⁶, C. Eduardo Corrales¹⁷, Tera M. Quigley¹⁶, Jonathon P. Whitton¹⁶, Vassili Valayannopoulos¹⁶

¹Harvard Medical School, ²University of Cambridge, ³University of California, ⁴Cincinnati Children's Hospital, University of Cincinnati, ⁵Medical College of Wisconsin, ⁶David Geffen School of Medicine UCLA, ⁷Nemours Children's Health, Jacksonville, ⁸University of Tübingen Medical Center, ⁹Columbia University Irving Medical Center, New York Presbyterian Hospital, ¹⁰University Clinic of Navarre, ¹¹GOSH Institute of Child Health, University College London, ¹²Hospital Ramón y Cajal, ¹³Nemours Children's Health, Orlando, ¹⁴Virginia Merrill Bloedel Hearing Research Center, Seattle Children's Hospital, University of Washington, ¹⁵Servicio de Genética, Hospital Universitario Ramón y Cajal, Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), ¹⁶Regeneron Pharmaceuticals, Inc., ¹⁷Brigham and Women's Hospital, Harvard Medical School

Background: Genetic deficiency of otoferlin (OTOF) disrupts synaptic transmission by the auditory sensory hair cells, causing congenital deafness. Children with OTOF-related deafness are typically treated with cochlear implants. DB-OTO is a gene therapy based on a dual adeno-associated virus 1 which delivers human OTOF complementary DNA regulated by a hair cell-specific promoter.

Methods: The CHORD trial is an open-label, single-arm, first-in-human registrational study evaluating DB-OTO (NCT05788536). Twelve children with profound deafness carrying biallelic OTOF pathogenic variants received an intracochlear DB-OTO infusion (7.2×10^{12} vector genomes/ear) unilaterally or bilaterally. The primary efficacy endpoint was the achievement of average thresholds ≤ 70 decibel hearing level (dBHL) by behavioral pure tone audiometry (PTA) at Week 24, a clinical standard that generally allows for natural acoustic hearing without the need for cochlear implants. The key secondary endpoint was the presence of auditory brainstem response to a 90 decibel normal hearing level (dBnHL) click stimulus. Incidence and severity of adverse events was a primary safety endpoint. Laboratory, limited vestibular, and immunogenicity testing were also performed.

Results: Following single DB-OTO administration, 11 of 12 participants (92%) with profound deafness at baseline were able to hear for the first time. Nine of 12 participants (75%) achieved clinically significant hearing improvements ($P < 0.0001$), enabling natural hearing, meeting the primary and key secondary endpoints, and obviating the need for cochlear implants. Six of 12 participants (50%) were able to detect soft speech without assistive devices (average PTA ≤ 45 dBHL), and 3 of 12 participants (25%) achieved complete normalization of hearing sensitivity (≤ 25 dBHL) by Week 24 as measured by average PTA. (Average PTA ≤ 25 dBHL). Preliminary data from 1.5 years of follow-up indicate sustained or improved hearing, as well as improvements in speech and language development. No DB-OTO-related adverse events were recorded.

Conclusions: In this first-in-human registrational gene therapy trial, DB-OTO resulted in significant hearing improvements in participants with OTOF-related deafness. DB-OTO administration enabled natural acoustic hearing for the first time, and normalized hearing sensitivity in some participants.

Marmoset Monkeys With a Non-Mosaic Disruption of the Otof Gene as a Model of Human Deafness

11:15 a.m. - 11:30 a.m.

Mara Uhl¹, Tobias Kahland², Dimitri Leonid Lindenwald², Marcus Jeschke³, Kathrin Kusch⁴, Olena Tkachenko Eikel², Nancy Rüger², Charis Drummer², Bettina Wolf⁴, Fritz Benseler⁵, Nils Brose⁵, Rüdiger Behr², Tobias Moser⁴

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Background: About 5% of the world's population suffers from disabling hearing loss. In newborns, 50% of hearing loss is attributed to genetic factors. One of the deafness genes is OTOF, causing the auditory synaptopathy DFNB9, which makes up 2-8% of congenital hearing loss. Within the last year's great progress has been made in gene therapy of DFNB9 from proof of concept in mice, to favorable biosafety data in non-human primates (NHP) and promising results in clinical trials. Yet questions of safety, efficacy, and longevity of different OTOF gene therapy strategies remain to be answered. Therefore, the need of a NHP model of DFNB9 is eminent. Moreover, such a model could be used to study other methods of hearing restoration, bridging the gap between mouse experiments and clinical trials.

Methods: Marmoset oocytes harvested upon ovary stimulation, were used for in vitro fertilization. Subsequently these fertilized oocytes were injected with Cas9 and four guide RNAs. At the 6 to 12-cell-stage the embryos were transferred to the uterus of surrogate mothers. After birth PCR genotyping of all germ layers was performed and the PCR products were subcloned to obtain single-clone DNA sequences. The phenotype of the animals was characterized, monitoring the weight development of the animals, analyzing their vocalizations and testing their hearing. Three to four vocalization recordings each lasting 10 min were performed at two to four weeks after birth. At 6 months of age, auditory brainstem responses to clicks and pure tones of different frequencies were probed and DPOAEs were acquired. Furthermore, immunohistofluorescence staining was performed on the organ of Corti of one of the OTOF-knockout marmosets.

Results: Two marmosets were born carrying biallelic mutations of OTOF, resulting in a knockout. They were born naturally and cared for normally by their parents. The OTOF knockout had no effect on the overall development. The mutations were detected in all germ layers, indicating the absence of mosaicism. Auditory brainstem recordings revealed a profound hearing loss, while the detection of otoacoustic emissions indicated the functionality of the cochlea upstream of synaptic sound encoding. The structural integrity of the organ of Corti was confirmed by immunohistofluorescence. Moreover, this method confirmed the loss of OTOF within the inner hair cells. Preliminary data suggest that the vocalizations of the deaf marmosets were largely unaltered compared to age-matched WT animals. They used the full vocal repertoire, and their vocal activity was similar to WT marmosets.

Conclusions: We generated OTOF-knockout marmosets via CRISP/Cas9 injection into in vitro matured and fertilized oocytes. OTOF-knockout marmosets present a non-mosaic NHP model for DFNB9. They provide a model for testing and optimizing safety, efficacy, and longevity of OTOF gene therapy and serve the development of other methods for hearing restoration such as optical cochlear implants.

SENS-601, a Novel Adeno Associated Vector-Based Gene Therapy as a Treatment for the Autosomal Recessive Non-Syndromic Deafness 1A (DFNB1A)

11:30 a.m. - 11:45 a.m.

Arnaud Giese^{*1}, Guillaume OLIVIER¹, Christophe Tranv Van Ba¹, Sandra Pierredon¹, Anne-Gabrielle Harrus¹, Pierre Rambeau¹, Lise Barrot¹, Anais Pages¹, Laurene Heriaud¹, Pauline Liaudet¹, Audrey Broussy¹, Selma Dadak¹, Christine Le Bec¹, Emilie Bousquet¹, Anne-Valérie

HERITIER², Charlene Vaux¹, Amrit Kaur ESTIVALET-SINGH², Charlene JOSEPHINE¹, Andrea Lelli², Muriel Sudres², Nicolas Michalski², Geraldine Petit¹, Rafik Boudra¹, Christine Petit², Laurent Desire¹

¹SENSORION SA, ²Institut de l'Audition/ Institut Pasteur

Background: The estimated prevalence of severe or profound deafness in humans is 1/1000 neonates, and genetic factors account for half of the cases. Pathogenic variants of GJB2, the gene encoding Connexin 26 (Cx26), are involved in 50% of congenital deafness and are mostly associated with autosomal recessive non-syndromic DFNB1A. In the cochlea GJB2, is largely expressed in the majority of supporting cells (SCs) of the neuroepithelium but not in sensory hair cells (SHCs).

Today, gene therapy strategies for autosomal recessive forms of deafness have shown, in clinical trials, promising hearing recovery. In some cases, some patients tend to show more rapid improvements in auditory and speech performance than those with cochlear implants. To this aim, we have developed SENS-601, an AAV vector for DFNB1A with proprietary cis-regulatory elements for a safe and targeted transgene expression. SENS-601 efficiently transduces human cell lines to produce Cx26 protein that is adequately addressed to the plasma membrane and allows functional gap junctions.

Methods: Several conditional mouse models were generated to elude embryonic mouse lethality caused by complete loss of Gjb2 expression, mimicking the most common forms of DFNB1A and severe/profound hearing loss and a deficit of the endocochlear potential. Intracochlear injections of SENS-601 lead to improvement of hearing thresholds as early as 3 weeks post-injection in a dose-dependent manner.

Results: Microscopic examination confirmed that SENS-601 efficiently and adequately restores Cx26 expression. Dose-response experiments show that intracochlear SENS-601 injection is safe over a wide range of doses administered and provides a significant therapeutic benefit in DFNB1A mice. Restoration of the endocochlear potential is currently being addressed.

Early tolerability and biodistribution of SENS-601, or SENS-601-FLAG, studies were conducted in mice and Non-Human Primates (NHP) after injections through the round window (RW). Transgene expression was observed in most cells that naturally express Gjb2 along the tonotopic axis of the cochlea, with good local and systemic tolerability. In particular, no SHCs express the transgene, confirming the specificity of the expression cassette in both species. Furthermore, SENS-601 long-term safety studies conducted in wild-type mice demonstrate persistence of transgene expression with no impact on auditory function or cochlear cytoarchitecture. High-dose intravenous injection of SENS-601 does not induce any adverse events or changes in behavior. A combined 3- and 6-month GLP toxicology and biodistribution study of SENS-601 is currently ongoing in NHP, following a single intra-cochlear injection administered using the surgical procedure and injection device intended for clinical use, to evaluate the local tolerability and safety of SENS-601.

Conclusions: Overall, efficacy, biodistribution and early safety data package support SENS-601 development to restore physiological hearing in DFNB1A patients

Capsid and Promoter Engineering for AAV Gene Therapy and Genome Editing in GJB2-Related Hearing Loss

11:45 a.m. - 12:00 p.m.

Kazusaku Kamiya^{*1}, Daisuke Arai¹, Hidekane Yoshimura², Shin-ya Nishio², Sho Kanzaki¹, Yutaka Takumi², Katsuhisa Ikeda¹, Shin-ichi Usami²

¹*Juntendo University Faculty of Medicine*, ²*Shinshu University School of Medicine*

Background: Mutation of GJB2 (encoding Gap Junction Beta 2) is the most frequent cause of hereditary deafness worldwide and accounts for up to 50% of non-syndromic sensorineural hearing loss. GJB2 encodes connexin 26, a component of cochlear gap junctions that help maintain ion balance in the cochlea. We previously demonstrated that GJB2 deficiency in mice impairs auditory function (Kamiya, J. Clin. Invest., 2014) and that immediate postnatal delivery of wild-type GJB2 to the inner ear via adeno-associated virus (AAV) vector restored the hearing loss in mice (Iizuka, Hum. Mol. Genet., 2015). However, delivery of GJB2 to the mature cochlea did not restore hearing. The early postnatal period in mice corresponds to the embryonic period of inner-ear developmental in humans. Thus, to develop a feasible clinical approach for human gene therapy, mature mice should be used for delivery of AAV to the inner ear. To achieve this, the development of an optimal AAV vector platform for GJB2-related hearing loss is indispensable.

Methods: We generated the several AAV capsids that effectively infect cochlear supporting cells by shuffling capsid sequences between wild serotypes or directed evolution with AAV library. And the promoters were modified to express only in GJB2 expressing cells and form proper gap junctions. Then the vectors were tested with adult GJB2 deficient mice (Kamiya, J. Clin. Invest., 2014) and our supporting cell model derived from human patient iPS (induced pluripotent stem) cells with typical GJB2 mutation (Fukunaga, Stem Cell Reports, 2016, Hum. Mol. Genet. 2021). As part of a preclinical study, we administered the novel AAV vector to non-human primates (NHPs).

Results: We generated novel AAV vectors by shuffling capsid sequences between wild serotypes and selecting those that effectively infect cochlear GJB2-positive cells. To avoid transgene expression in non-target cells such as hair cells, we developed a specific promoter for GJB2-expressing cells. Novel AAV-mediated delivery of GJB2 significantly restored hearing loss in mature GJB2-deficient mice, and gap junction recovery was confirmed in both animal (GJB2-deficient mice) and cellular models (patient iPS cell-derived models). In NHPs administered with our novel AAV vector, AAV-derived protein expression in the target cells (cochlear supporting cells and fibrocytes) was broadly detected.

Conclusions: These findings indicate that our AAV vector carrying the novel capsid/promoter could serve as an optimal platform for gene therapy of GJB2-related hearing loss.

Dual AAV Gene Therapy Achieves Sustained Recovery of Hearing and Auditory Processing in a DFNB16 Mouse Model

12:00 p.m. - 12:15 p.m.

In Vivo Evolved AAV Variants Enable Dual-AAV Stereocilin Delivery and Outer Hair Cell Rescue

12:15 p.m. - 12:30 p.m.

Josephine Wilkes¹, Jennifer Marx¹, Nico Jaeschke¹, Lisa Prager¹, Sabrina Just¹, Philipp John-Neek¹, C Wiehlmann¹, A Gardenstaeeter², Matthias Gerlitz², Erdem Yildiz², Athanasia Warnecke¹, Odett Kaiser¹, Hinrich Staecker^{*3}, Hildegard Buening¹

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Background: Hearing loss (HL) affects ~20% of the global population, yet current interventions are limited to hearing aids and cochlear implants. Gene therapy offers a promising alternative by directly addressing the molecular causes of HL. Adeno-associated virus (AAV) vectors are powerful gene transfer tools, but most variants used in the inner ear originate from libraries selected in other tissues or in silico designs. Directed evolution in the inner ear can generate optimized capsids with higher tropism for auditory cell types. This is particularly relevant for DFNB16, the second most common cause of autosomal recessive non-syndromic HL, resulting from mutations in the stereocilin (STRC) gene. The large size of STRC necessitates dual-AAV strategies, such as RNA trans-splicing systems, to enable full-length gene delivery.

Methods: AAV peptide display libraries based on AAV1, AAV2, and AAV6 with random 7-mer insertions in capsid region VIII were screened in vivo in adult mice (n=4/library) using alternative administration routes, requiring vectors to overcome robust biological barriers. High-throughput sequencing identified enriched capsids, which were produced as vectors carrying a dTomato reporter and tested in mice and pigs. For therapeutic application, the most promising OHC-targeting capsid (Var1) was employed in a dual-AAV system encoding split STRC genomes (dual-AAV1.Var1). Strc-deficient mice were treated at one month of age, and auditory brainstem responses (ABRs), stereocilia morphology, and transgene expression were assessed up to six months post-treatment.

Results: Selection enriched distinct capsids, with AAV2-derived variants accumulating up to 5% and AAV1-derived up to 2.5%. Top variants showed improved transduction compared to parental serotypes, including efficient targeting of outer and inner hair cells, stria vascularis, and spiral ganglion neurons (SGNs). In pigs, two lead candidates (Var1, Var3) reproducibly transduced cochlear cells. Using Var1 in a dual-AAV strategy, full-length STRC expression was confirmed in vitro within 48 h. In vivo, treated Strc-deficient mice showed modest ABR threshold improvements: small recovery at 16 kHz after 7 weeks and significant shifts across all tested frequencies at 6 months, though thresholds remained above 60 dB, indicating moderate-to-severe HL. In contrast, untreated ears showed severe-to-profound HL (> 80 dB). Whole-mount imaging revealed partial stereocilia recovery on 50–80% of OHCs in treated ears, and FLAG immunostaining confirmed STRC expression in OHCs, with additional expression in SGNs and spiral ligament, likely reflecting mRNA regulatory sequences.

Conclusions: This work establishes a panel of inner ear-optimized AAV variants with distinct tropisms and demonstrates the first in vivo application of dual-AAV STRC system in mature Strc-deficient mice. While hearing recovery was partial, the combination of OHC transduction, stereocilia rescue, and stable STRC expression validates the therapeutic potential of dual-AAV

delivery for DFNB16. These findings highlight the feasibility of tailoring AAV capsids for the inner ear and advance gene therapy approaches for large-gene hearing loss disorders.

Young Investigator Symposium 1: Real-World Acoustic Environments and Their Impact on Hearing and Well-Being

10:30 a.m. - 12:30 p.m.

Meeting Room 208ABC, Floor 2

Real-World Acoustic Environments and Their Impact on Hearing and Well-Being

Erika Skoe, *University of Connecticut*

Emily Spitzer, *New York University Grossman School of Medicine*

The Daily Auditory Environments of People With Tinnitus

Erika Skoe, *University of Connecticut*

Individual Abstract: Background:

Tinnitus is the sensation of sound in the absence of an external stimulus. The connection between long-term noise exposure and tinnitus has been the target of many studies, but little attention has been given to noise exposure patterns after the onset of tinnitus. A deeper understanding of the auditory environments of people with tinnitus is needed to aid the clinical management of tinnitus.

Methods:

For a week, participants (n=108) wore a personal noise dosimeter during all waking activities to measure their daily noise exposure. Participants also kept a journal of their activities during the week. The total sample size includes both tinnitus (n=61) and control groups (n=56). Dosimeter data were analyzed first using summative metrics of daily sound exposure, followed by functional data analysis (FDA) to reveal finer temporal differences in sound exposure between groups. The journal data were coded into 17 different categories to identify activities associated with noisy periods in the dosimeter record (> 85 dBA) and to compare groups with respect to their activity levels.

Results:

We found that daily personal noise exposure, measured using the time-weighted average sound pressure levels (LAeq,8h), was lower for the Tinnitus group relative to the Control group. Based on the dosimeter data, neither group was at ongoing risk of noise-induced hearing loss, as

average daily sound levels did not exceed 75 dB LAeq,8h for either group. Yet, the Tinnitus group spent less time engaged in noisy activities (> 85 dBA) compared to the Control group, with notable differences for home and workplace settings. However, overall, the groups did not differ in the total overall time they spent in these settings during the week of dosimetry. The FDA revealed that group differences were maximal around noon and evenings, periods when an individual is likely to have more control over their environment.

Conclusions:

Overall, we find that people with tinnitus tend to have quieter environments, suggesting that they lead quieter lives. Differences between groups may be explained by greater awareness of the risks of noise exposure in the tinnitus group or decreased sound tolerance among this group. Ongoing work seeks to replicate findings using smart Watch technology. One of the primary advantages of smart Watch technology is that, unlike personal noise dosimeters, it can provide data on headphone usage. Preliminary data will be presented.

Cultural Differences in Listening Environments Between Hispanic and White Non-Hispanic Cochlear Implant Users

Emily Spitzer, *New York University Grossman School of Medicine*

Individual Abstract: Introduction: Cochlear implant (CI) speech processors can track usage via “data logging”, including the number of hours on air, time spent in different acoustic environments and loudness levels. These data are important, as high noise levels and poor signal to noise ratios (SNRs) can decrease speech intelligibility, impairing communication and speech perception outcomes. Louder environments can have a detrimental impact on learning and cognition and have been associated with both socioeconomic disparity and cultural factors. A study found that normal hearing Latinx-identifying college students experienced higher levels of environmental noise and lower SNRs, possibly due to cultural importance of collectivism and music listening at louder levels. This study investigates whether similar listening patterns exist in CI users and examines how data logging variables relate to speech perception outcomes.

Methods: Retrospective chart review of CI patients at a tertiary medical center in New York City identified 30 Hispanic and 31 White non-Hispanic adults for further review. Demographic variables were compiled and home zip codes were used to obtain population-based socioeconomic data. Outcome measures included speech perception scores (in noise and quiet) conducted in the participant’s primary language and data logging information extracted from the CI speech processor. The datalog included hours of total use and time spent in different listening environments, classified by the CI software into sound levels (in dBA) and sound scenes (noise, quiet, speech, music, and “other”).

Results: There were no significant differences detected in total hours of CI usage, age at CI, or gender between the Hispanic and White non-Hispanic groups. There was a significant difference in the percentage of time spent in each scene: Hispanic Americans spent more time in “speech in noise”, “music”, and “noise”; non-Hispanics spent more time in “quiet”. Hispanic participants lived in neighborhoods with higher population densities, which correlated to higher CI sound

levels (dBA > 70) in the environment. The percentage of time spent in noise positively correlated with sentence-in-noise perception scores for Hispanic participants, but not for White non-Hispanic participants.

Conclusion: Cultural differences appear to exist between Hispanic and White non-Hispanic listening environments, like normal hearing listeners. While device use was similar, the Hispanic group had greater noise exposure, which was linked to better speech-in-noise performance. This relation may suggest an auditory training mechanism, reinforced by cultural values. Audiologists should consider cultural background and listening preferences when counseling CI users, as these may influence performance and adaptation to noisy environments.

Linking Hearing Ability, Acoustic Environments, Hearing Aids, and Social-Emotional Health Outcomes

Erik Jorgensen, *University of Wisconsin-Madison*

Individual Abstract: Background

Hearing loss is linked to increased loneliness and depression, but the mechanisms behind this association remain unclear. A leading hypothesis is that hearing loss leads to social-emotional difficulties by changing hearing-related behaviors—particularly by prompting people to avoid speech-in-noise (SiN) environments, which are often social and especially challenging for individuals with poor speech perception in noise (SPIN). This study tested that hypothesis by examining the relationships among hearing, hearing aid use, hearing-related behaviors, and social-emotional health.

Methods

The study included 54 younger adults with typical hearing, 55 older adults with hearing loss who did not use hearing aids, and 52 older adults with hearing loss who did. Participants completed standardized measures of SPIN, depression, and loneliness. They also wore body-worn recorders near the head for all waking hours for one week (younger adults) or two weeks (older adults). A deep neural network classified the acoustic environment in 0.5 s intervals, and the percent of time in each environment was computed. Descriptive statistics and structural equation modeling were used to analyze moderated mediation models linking acoustic environments, SPIN, loneliness, and depression.

Results

Younger adults spent the most time in SiN environments. Surprisingly, older hearing aid users spent significantly less time in SiN and more time in silence compared to non-users. Among younger adults, more time in SiN was associated with lower loneliness, especially for those with better SPIN, which indirectly reduced depression. For older non-users, a similar pattern emerged: more SiN exposure was associated with lower loneliness, especially with better SPIN. In contrast, for hearing aid users, more time in SiN was linked to greater loneliness. SiN exposure and SPIN ability were not associated with depression in older adults. An “environment-person fit” effect was also observed: those with poor SPIN who spent less time in SiN had lower

loneliness. Among older adults, time in SiN, SPIN ability, hearing aid use, and loneliness were all related to perceived hearing-related disability.

Conclusions

Acoustic environments vary by age and hearing status, with implications for social-emotional health. In younger adults, SPIN moderates the link between SiN exposure and loneliness, which in turn affects depression. Among older adults, hearing aid use does not mitigate loneliness or increase time in SiN; hearing aid users had poorer SPIN, spent less time in SiN, and reported higher loneliness than non-users. Poor SPIN and perceived hearing disability likely underlie avoidant hearing-related behaviors, pursuit of hearing aids, and loneliness.

Acoustic Environments for Children Who Are Deaf or Hard of Hearing

Carlos Benitez-Barrera, *University of South Florida*

Individual Abstract: Objectives:

Children who are Deaf or Hard of Hearing (CDHH) who develop spoken language are at increased risk for language delays. These delays are influenced by hearing-related factors (e.g., age of amplification) and reduced exposure to caregiver language input. Emerging models of language acquisition emphasize that environmental noise also limits access to caregiver input, further impacting language development. Our previous work showed that real-world speech-to-noise ratios (SNRs) predict language outcomes in children with typical hearing, beyond the effect of caregiver talk. Building on this, we propose that CDHH exposed to both limited input and poor acoustic access face the highest risk for language delays.

This presentation underscores the importance of assessing the quality of CDHH children's acoustic environments. Specifically, we highlight: (1) how wearable audio recording devices can objectively quantify children's auditory experiences, including access to caregiver input, and (2) how these experiences relate to language development.

Design:

We are recruiting 40 preschool CDHH (ages 3–6) and an age-matched control group with typical hearing. Our goal is to develop and validate a novel Speech Accessibility Index (SAI), which quantifies children's access to spoken language in everyday settings. The SAI integrates two metrics: (a) each child's Speech Reception Threshold (SRT) in noise, and (b) SNRs calculated from daylong LENA recordings collected at home. The SAI estimates the percentage of conversations with SNRs above the child's SRT, serving as a proxy for real-world language access. This approach builds on our prior work and extends conventional measures of language exposure to include the quality of acoustic input available to the child.

Results:

To date, we have collected data from 20 children (10 CDHH). Preliminary analyses indicate that both caregiver input and access to language (as indexed by the SAI) are significantly associated with language outcomes when combining both groups. We expect that, with the full dataset, both predictors will remain independently associated with language skills, even after controlling for relevant covariates.

Conclusions:

The SAI captures dimensions of children's language environments that go beyond caregiver speech alone, offering clinical value in assessing real-world speech access. It shows promise as a screening tool for identifying CDHH at risk for language delays and for informing home-based interventions (e.g., remote microphone systems) aimed at enhancing children's language access and outcomes.

Work supported by NIH-NIDCD (R21 DC022038) to C.R. Benítez-Barrera

Poster Session II with Coffee

1:30 p.m. - 3:00 p.m.

Ballroom A, Floor 3

Due to the policies of the Puerto Rico Convention Center, the Poster Hall will be available to attendees between the hours of 7AM – 11PM (local time), with a 1- hour break every day between 11AM and 12 PM (Noon) to facilitate setup for the next poster session. This is an appreciated extension of their normal permitted hours. ePosters are available on the conference website and the ARO MWM mobile app. and nbsp;

SU1. Silence and Solitude: Effects of Noise Exposure and Social Isolation on Serotonergic Function and Behavior

Megan Walker¹, Jessica Bush¹, Lauren Hadland¹, Laura Hurley*¹

¹*Indiana University*

Background: The social isolation that can accompany hearing impairment in aging co-occurs with other health concerns, including depression, anxiety, and cognitive decline. A contributing factor to these negative outcomes may be dysfunctional communication driven by deficits in auditory processing and perception. Animal models of these related risk factors can help elucidate the neural mechanisms underlying these functional challenges. Serotonin-auditory interactions are responsive to both hearing loss and social isolation in mice.

Methods: We therefore assessed the effects of noise exposure and a period of individual housing on serotonergic function and social and open field behaviors in female CBA/CaJ mice, using a factorial design. At 6 weeks of age, mice were exposed to noise from 8-16 kHz at 100 dB, or to sham treatment, for 2 hrs. At 11 months, mice were placed in individual housing for 4 weeks or maintained in social groups, after which they were placed in an opposite-sex social interaction with socially housed male partners. Following brain fixation and slicing, sections were labeled with antibodies for tryptophan hydroxylase plus c-Fos, or the serotonin transporter (SERT).

Results: Social isolation had strong effects on behavior, elevating vocal behavior in both the female subjects and their male partners, and increasing time in the edges of the open field. In contrast to previous studies in younger mice, social isolation did not alter the density of SERT+ fibers in the inferior colliculus or neurons double labeled for c-Fos and TPH in the dorsal raphe nucleus (DRN). Although noise exposure significantly elevated ABR thresholds, it did not affect vocal behavior or SERT+ density. Noise exposure did increase the density of neurons double

labeled for c-Fos and TPH in the dorsal raphe nucleus. However, the effect of noise was significant only in mice in the isolated treatment group. Further, the density of double labeled neurons in the DRN correlated with ABR threshold and with USV number.

Conclusions: These findings suggest that noise exposure and social isolation interact to influence the activity of serotonergic neurons during social interaction.

SU2. How Reverberation Shapes the Subcortical Encoding of Speech in Aging and Hearing Loss

Ebtesam Sajjadi*¹, Samira Anderson¹

¹*University of Maryland*

Background: Everyday communication often occurs in environments with reverberation, such as classrooms, restaurants, and auditoriums. Mild reverberation levels can enhance overall speech audibility, but excessive levels of reverberation blur temporal and spectral cues and deteriorate speech understanding. This difficulty is more pronounced for older adults and individuals with hearing loss. The purpose of this study was to examine how reverberation affects subcortical speech encoding to gain a better understanding of factors that may contribute to perceptual declines.

Methods: The current project uses frequency-following responses (FFRs) to investigate the subcortical neural encoding of reverberation in older adults. Prior work has shown that age-related declines in temporal precision particularly compromise encoding of dynamic formant transitions in speech, whereas steady-state vowel segments may be relatively preserved. Therefore, FFRs have been recorded to the syllables /da/ and /ah/ to represent dynamic (formant transition) and static (steady-state vowel) components of speech, respectively. Stimuli are presented under no reverberation, short decay (RT60 = 0.46 s), and long decay (RT60 = 2s) conditions. FFRs have been recorded from older adults with normal hearing (ONH) and mild to moderate hearing loss (OHI) at 80 dB SPL. Responses are analyzed using phase locking to the envelope and temporal fine structure and stimulus-to-response correlations to assess the fidelity of neural encoding across different time regions of the speech signal.

Results: Preliminary results show that reverberation reduces phase locking and decreases stimulus-to-response correlations for both /da/ and /ah/ stimuli. Early findings suggest that long decay reverberation particularly weakens encoding of the transition region in /da/. This result is consistent with the previous findings that dynamic cues are more vulnerable to temporal smearing. While no statistically significant group effects have yet been observed, OHI participants generally show weaker envelope encoding under reverberant conditions than ONH participants.

Conclusions: These findings provide initial evidence that reverberation exacerbates age-related declines in neural precision, particularly for dynamic speech regions. Ongoing data collection will provide evidence about the differential effects of reverberation on steady-state and transition regions, and whether OHI listeners are at greater risk for degraded neural synchronization. This work aims to increase our understanding of how reverberation shapes subcortical encoding in aging populations and propose future strategies for improving the ability of this population to understand speech in acoustically complex environments.

SU3. Vascular Senescence and Microcirculatory Pathology as Contributors to Age-Related Hearing Loss

Marta Konopka^{*1}, Maria Luque¹, Rudolf Glueckert¹, Elisabeth Pechriggl¹, Erich Brenner¹

¹*Medical University of Innsbruck*

Background: Age-related hearing loss (ARHL) is one of the most prevalent sensory disorders in the elderly and is closely associated with cognitive decline and reduced quality of life. Increasing evidence suggests that vascular dysfunction, including endothelial alterations, chronic low-grade inflammation, and systemic vascular remodeling, contributes to cochlear degeneration. Human-based studies are required to clarify these mechanisms in the context of aging and diabetes.

Methods: Human postmortem specimens from donors older than 60 years were analyzed, including cochlea, internal carotid artery, kidney, and skin. Donors with type 2 diabetes mellitus were compared with non-diabetic controls. Tissues were processed using conventional histology and immunohistochemistry to assess four domains: vascular aging, low-grade inflammation, microangiopathies, and macroangiopathies. For this purpose, α -smooth muscle actin (α -SMA) was used to evaluate vascular smooth muscle remodeling, CD31 to assess endothelial integrity and vascularization, and CD68 to detect macrophage infiltration. Quantitative image analysis was applied where tissue quality allowed, enabling comparison of vascular remodeling, endothelial changes, and inflammatory burden across diabetic and non-diabetic groups.

Results: Macrovascular remodeling in the carotid artery was characterized by thickened media and pronounced intimal hyperplasia, consistent with advanced atherosclerotic change. Microvascular alterations were observed in cochlear and systemic tissues, including focal basement membrane irregularities and altered vascular density. A trend toward higher CD31 expression was detected in diabetic donors compared with non-diabetic controls, likely reflecting compensatory endothelial proliferation and remodeling in the setting of diabetic vasculopathy. Inflammatory infiltration was consistently observed, with CD68-positive macrophages located in arterial walls and cochlear regions. Quantification demonstrated higher macrophage infiltration in diabetic donors, supporting the association between chronic low-grade inflammation and vascular pathology. These findings currently represent trends, but with a larger donor database we expect not only confirmation of these patterns but also significant differences to emerge, clarifying the extent of vascular involvement in ARHL. Comparable alterations in kidney and skin underline the systemic nature of these changes.

Conclusions: By integrating inflammatory, microvascular, and macrovascular findings across cochlea and systemic tissues, this study supports the concept that ARHL represents a manifestation of systemic vascular aging rather than an isolated disorder of the inner ear. All findings will be correlated with progression of age-related hearing loss, providing a translational framework that links vascular pathology to auditory decline. These results highlight vascular dysfunction as a promising therapeutic target and emphasize the need for preventive strategies aimed at maintaining vascular integrity to preserve auditory function in aging and metabolically compromised populations.

SU4. Oncomodulin Attenuates Oxidative Stress-Induced Senescence in Auditory HEI-OC1 Cells

Janith Nilmika Halpage Hewa Halpage*¹, Santha Ranganathan¹, Ian Stahl¹, Dwayne Simmons¹

¹*Baylor University*

Background: Age-related hearing loss (ARHL) is characterized by the progressive dysfunction/loss of cochlear outer hair cells (OHCs), yet the molecular mechanisms contributing to their decline remain unclear. Cellular senescence, a permanent state of cell cycle arrest triggered by stress, is increasingly recognized as a contributor to age-related tissue degeneration including in the auditory system. Oncomodulin (OCM), a calcium-binding protein highly expressed in OHCs, is essential for calcium homeostasis. The hypothesis for this project is that the loss of OCM accelerates OHC senescence in auditory cells.

Methods: To investigate the potential role of OCM in regulating stress-induced senescence, we used the HEI-OC1 cochlear cell line and induced senescence using hydrogen peroxide (H₂O₂). Cells were transfected with plasmids encoding OCM (fused to GFP or mCherry). Senescence-associated β -galactosidase (SA- β -gal) and p16INK4a immunofluorescence were looked at to measure levels of senescence. To investigate the relationship between autophagy and senescence, immunofluorescence analysis was performed using antibodies against LC3B and p62.

Results: OCM-expressing cells exhibited significantly reduced senescence-associated β -galactosidase (SA- β -gal) activity compared to controls, along with decreased expression of the senescence marker p16INK4a. Morphological analysis showed increased nuclear enlargement, a characteristic feature of senescent cells, in control cells compared to OCM-transfected cells. Further investigation revealed that OCM modulates autophagic signaling. Immunofluorescence analysis of LC3B and p62 puncta demonstrated decreased autophagic stress in OCM-expressing cells, suggesting reduced autophagic flux. Additionally, gene expression analysis showed downregulation of p53, key regulator of the senescence pathway. Notably, mitochondrial DNA expression level was elevated in OCM-transfected cells, indicating enhanced mitochondrial integrity and resilience to oxidative stress.

Conclusions: These findings suggest that OCM suppresses cellular senescence in cochlear cells through the preservation of autophagic function and maintenance of mitochondrial health. This work identifies increased calcium buffering as a novel protective factor and highlights its potential relevance in mitigating ARHL.

SU5. Impact of Auditory Training on Cortical Neural Tracking of Rapid Speech

Ciaran Stone*¹, Ebtesam Sajjadi¹, Samira Anderson²

¹*University of Maryland, College Park*, ²*University of Maryland*

Background: Age-related hearing loss, and aging itself, can make understanding rapid speech challenging, as degraded processing in the auditory system limits the ability to process temporal cues. Current research suggests that these difficulties are linked to changes in the brain's temporal dynamics, including delayed and exaggerated responses to speech, which may represent compensatory mechanisms that often fail to fully restore speech perception. This

study's main goal is to explore whether auditory training with time-compressed speech can improve both behavioral performance and cortical processing, and if these improvements can be observed across different hierarchical levels of neural representation.

Methods: In this study, 94 participants were recruited: a younger normal-hearing (YNH) group, an older normal-hearing (ONH) group, and an older hearing-impaired (OHI) group. Each participant underwent pre- and post-training sessions. During these sessions, a behavioral assessment was conducted to determine each participant's threshold for 50% keyword recognition of time-compressed speech. Electroencephalography (EEG) was used to record cortical envelope tracking of continuous speech of audiobooks in both unprocessed and time-compressed conditions. All participants completed six adaptive training sessions where the time-compression rate was adjusted based on their performance.

Results: Our findings showed a significant improvement in behavioral measures after training. Both the ONH and OHI groups demonstrated better performance at the post-test. Notably, while the younger listeners still had the highest overall performance, the post-training thresholds for the ONH group approached the pre-training levels of the YNH group. These results suggest that this targeted training can partially restore rapid speech recognition, especially in older adults with normal hearing. To further understand the neural basis of these improvements, the cortical EEG data will be analyzed using a Temporal Response Function (TRF) framework to evaluate changes in neural encoding at various levels, from sensory (e.g., acoustic features) to linguistic (e.g., phonemes, words, phrases, and sentence onsets). We will present how the exaggerated cortical tracking responses previously observed in older adults change with training.

Conclusions: Our findings provide new and important insights into the potential for auditory training to improve rapid speech perception in older adults, and perhaps for their speech perception in general. The behavioral results suggest a partial restoration of function, which has impactful clinical relevance for aging adults. This type of targeted training could provide a valuable tool to help individuals overcome challenges with speech comprehension in everyday situations. Future research may investigate the long-term effects of such training and its potential application in home and clinical settings. A key takeaway is that auditory training can drive beneficial neuroplastic changes that improve speech recognition, especially in older adults with normal hearing. This work was supported by NIA P01AG055365.

SU6. Maturation of Cortical Auditory Evoked Potentials in Response to Tones and Noise

Bruna Mussoi^{*1}, Julia Huyck²

¹*University of Tennessee Health Science Center*, ²*Kent State University*

Background: Brain structure and function undergo important changes during adolescence. Although the most significant neuroplastic changes happen during the first few years of life, cortical auditory evoked potentials (CAEPs) and auditory perceptual abilities do not become adult-like until early adulthood. The few studies that have investigated maturational changes in CAEPs during adolescence focused on a single type of stimulus, such as clicks, pure tones, or speech stimuli. However, given prior research indicating that children and adolescents may be more susceptible than adults to the negative effects of background noise during speech perception tasks, a comparison of CAEPs to tones vs. noises might yield valuable information

about the auditory encoding underlying these perceptual differences. Thus, the goals of this study were to examine (1) maturational changes in CAEPs to tones and noise, and (2) the relationship between CAEPs and speech recognition in noise in adolescents and young adults.

Methods: Ninety-four adolescents and young adults (10 – 23 years old) took part in this study. CAEPs were obtained in response to 400 ms long, 1000 Hz pure tones and band-pass noise, presented at 70 dB SPL via an insert earphone to the left ear. 800-1000 responses were recorded using surface electrodes (3-channel) and band-pass filtered between 1-30 Hz. Peak latencies and amplitudes (relative to 100 ms pre-stimulus baseline) were compared as a function of age. Speech perception in noise was assessed with the QuickSIN test.

Results: CAEP morphology differed in response to tones and noise, in that responses to noise stimuli had smaller N1 amplitudes and larger P2 and N2 amplitudes. From early adolescence to young adulthood, P1 and N1 latencies decreased in response to both tones and noise. In contrast, P2 latencies increased (tones only), as did N2 latencies (both tones and noise). P1 and N1 amplitudes tended to decrease from adolescence to young adulthood, while P2 and N2 amplitudes increased. Speech perception in noise improved during the course of adolescence, but this improvement did not appear to be associated with CAEP latencies and amplitudes to tones or noise.

Conclusions: There were extensive changes in CAEPs in the period between early adolescence and young adulthood. Results confirmed previous findings of continued maturation of CAEPs during adolescence and extend these findings in response to noise stimuli. CAEP responses to noise were markedly different from responses to tones, but results did not indicate a differential impact of age during adolescence. Other factors not examined in this study may be able to better predict maturational effects of adolescence on speech recognition in noise.

SU7. Cortical Representation of Attended and Ignored Acoustic Features Under Binaural Unmasking

Madeline Johnson*¹, Zilong Xie¹

¹*Florida State University*

Background: Binaural unmasking refers to the auditory system's ability to utilize interaural differences to segregate a target sound from background noise. This mechanism has been linked to enhanced cortical encoding of the acoustic features of attended continuous speech. Interestingly, even when background noise is not the focus of attention, its acoustic features still manifest in cortical responses. These observations raise an important question: To what extent does binaural unmasking influence the cortical representation of ignored auditory input during continuous speech processing in noise?

Methods: Twenty young adults with clinically normal hearing participated in a listening task involving audiobook stories embedded in speech-shaped noise at a signal-to-noise ratio of -2 dB. To examine the effects of binaural unmasking, two listening conditions were employed: S0N0, where both speech and noise were presented in-phase across ears, and S π N0, where speech was delivered out-of-phase while noise remained in-phase. EEG recordings were collected throughout the task. After each trial, participants responded to comprehension questions and provided subjective ratings on how well they grasped the story's gist and the effort required to understand it. Cortical tracking of acoustic features was analyzed using multivariate temporal

response functions (mTRFs), which modeled cortical EEG responses from spectrotemporal features (envelope and acoustic onsets) of both the target speech and the noise.

Results: Compared to the S0N0 condition, the S π N0 condition yielded enhanced behavioral performance, including higher accuracy in story comprehension, stronger ratings for gist understanding, and reduced perceived effort during listening. At the neural level, cortical TRFs to both envelope and acoustic onsets of the target speech were stronger and occurred earlier in the S π N0 (vs. S0N0) condition. Moreover, the mTRF models showed higher predictive accuracy for the target speech in the S π N0 (vs. S0N0) condition when using combined acoustic features or acoustic onsets alone. Finally, although cortical tracking of acoustic features of the noise was observed, no significant differences in TRFs or model prediction accuracy were found between the two listening conditions.

Conclusions: These findings reveal an interaction between top-down selective attention and binaural unmasking in cortical processing of continuous speech in noise. Future work should explore this interplay in multi-talker conditions.

SU8. Electroencephalographic Correlates of Auditory Selective Attention in Hearing Loss

Sharadhi Umesh Bharadwaj^{*1}, Olivia Flemm², Shaina Wasileski², Hari Bharadwaj²

¹*Carnegie Mellon University*, ²*University of Pittsburgh*

Background: Current clinical practices for sensorineural hearing loss (SNHL) emphasize audibility, and provide amplification to compensate for elevated audiometric thresholds. Yet, even with amplification, many patients with SNHL struggle to understand speech in noisy environments. Although most research on SNHL focuses on sensory deficits, relatively little is known about how top-down selective attention processes may be altered in individuals with hearing loss. To investigate this, we simultaneously record 32-channel EEG and behavioral responses.

Methods: Simultaneous 32-channel EEG recordings and behavioral measurements data has so far been collected from 8 subjects with normal hearing and 14 subjects with mild to moderate hearing loss. The matrix sentence test material was used to create a speech-on-speech task where the target and masking streams were constructed using the same group of words in the same speaker's voice at 0 dB target-masker ratio. The target and masker streams are lateralized to opposite hemifields using interaural time differences and staggered in time. This design minimizes acoustic overlap and thus reduces peripheral sensory interference, allowing our measures to primarily reflect top-down attentional processes rather than peripheral deficits. We examined two EEG correlates of spatial attention, (1) lateralized alpha-band oscillations (7–15 Hz) in parietal and occipital regions which reflect endogenous attention allocation during the preparatory phase, and (2) enhanced evoked potential amplitudes which reflect how the top-down attention modulates the sensory representation under focused attention. Stimuli and target location were counterbalanced across trials.

Results: To quantify alpha lateralization, we used the multitaper technique to compute alpha band power over parieto-occipital channels, comparing power between 'attend-left' and 'attend-right' trials. Attentional modulation was quantified by computing evoked potentials to stimulus onset and comparing the amplitudes between 'attend-lead' and 'attend-lag' trials.

Preliminary results suggest reduced alpha lateralization in individuals with hearing loss and trends of lower attentional modulation of sensory stimulus.

Conclusions: These data (if trends hold) would provide evidence that top-down attention is also altered in SNHL over-and-beyond sensory deficits that are known to exist. Our ongoing work aims to expand our dataset and characterize how top-down attentional control is affected in SNHL, shedding light on the interplay between sensory deficits and higher-order processes.

SU9. Probing Invariances in Auditory Event Categorization Using Model Metamers

Hee So Kim^{*1}, Malinda McPherson-McNato², Abigail Noyce¹, Jenelle Feather¹

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Background: Real-world acoustic inputs contain rich sensory information that we parse into discrete auditory objects and categories. However, the transformation from acoustic waveforms to neural representations is complex and poorly understood, especially as neural representations are invariant across some feature changes, such as variation in the exact pitches or timing. Further, previous investigations of auditory category representations have largely explored speech, music, or tone structures, with very few studies exploring categorical representations of non-speech stimuli. Computational modeling allows researchers to explicitly probe the transformation from input waveforms into higher-level representations, with recent work suggesting that deep neural networks (DNNs) are well-suited to model human auditory processing. Here, we test whether the invariances of DNNs trained to categorize non-speech sound stimuli are aligned with the invariances of human observers.

Methods: We developed an experimental paradigm to compare humans and DNNs using forced-choice classification across 25 non-speech sound categories using sounds obtained from the FSD50K dataset. For the tested models, we generated ‘model metamers’—stimuli whose activation produces the same response as the natural stimulus at a given model stage. We then tested whether humans (n=4) could recognize the model metamers as well as they could recognize the original audio. Recognizable model metamers imply that invariances of the model are shared with those of human observers. We tested DNNs pretrained on three different tasks: (1) word recognition only, (2) auditory event recognition only, and (3) a multi-task model for word recognition, speaker recognition, and auditory event recognition. These models were trained to be robust to small “adversarial” perturbations in the input cochleagram, a technique that was previously shown to help align the invariances of models with the invariances of human observers in the speech domain. For comparison of including this type of training, we also tested a multi-task model without adversarial training.

Results: First, humans and models can reliably categorize the natural sounds, demonstrating that our novel behavioral paradigm is well suited for testing invariances in auditory categories. For all models, we found that recognition accuracy for model metamers dropped significantly, but there was variability across models. Metamers generated for the adversarially trained auditory event recognition model were the most recognizable among the four models. This result indicates that task-specific optimization plays a significant role in creating human-like invariances. Among the multi-task models, metamers from the adversarially trained model had significantly

higher accuracy, confirming that training with adversarial perturbations helps align model invariances more closely with those of humans.

Conclusions: The results indicate that model metamers provide a powerful tool for probing human auditory perception in the domain of categorical representations of auditory events. These findings provide additional evidence that adversarial training and task-specific optimization can improve model alignment with human perception.

SU10. Neuronal Correlates Underlying Auditory Categorization in Mice

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Background: Categorization of sensory stimuli is a fundamental computation in cognition, and is critical for many aspects of learning, perception, and prediction. Auditory categorization (AC) has been linked to the neuronal activity in the auditory cortex, however, it remains poorly understood how categorical representations emerge in population neuronal activity with learning.

Categorization is often influenced by prior information and experience and can be modified by new information. We recently showed that during categorization learning, mice use multiple complementary strategies (Collina et al., 2025). These strategies affect the animals' perception of stimuli category under uncertain conditions, e.g., for stimuli that surround the categorization boundary. Additionally, animals modify these strategies based on their training. To understand neuronal correlates of auditory categorization, we recorded neuronal activity from the auditory cortex of mice using implanted high-density electrodes while training them to perform an auditory categorization task.

Methods: Mice were implanted with custom-made chassis housing Neuropixels electrodes in the auditory cortex and trained to discriminate between high and low frequency tones in 2-alternate forced choice (2AFC) task. Once their behavioral performance reached criterion %, we tested how well population neuronal activity categorized ambiguous tones around the categorization boundary, and how this boundary was affected by biasing the distribution of the stimuli toward high or low frequency tones.

Results: Single neuron analysis indicated that approximately 30% of the recorded neurons exhibited category preference, as measured by the difference in the firing rate of neurons was for stimuli between categories than within categories. Additionally, population decoding accuracy to between categories stimuli increased with training. To examine how neuronal population reflect behavior, we compared the neurometric and psychometric thresholds across sessions. Training increased alignment between thresholds of neurometric and psychometric curves with well aligned threshold estimates in fully trained animals. Moreover, a subpopulation of neurons was choice-selective, as their firing rate reflected the choice of the mouse across trials. Additionally, we also identified components in the population responses that signaled reward prediction, reward response and reward prediction error in AC.

Conclusions: Our results show that neuronal activity in the auditory cortex during auditory categorization reflects behavioral performance. We are currently analyzing how the neuronal

population activity evolves throughout auditory learning and how biasing the category distribution across different sessions affects neuronal activity.

SU11. Reduced Sound Tolerance due to Enhanced Central Gain in a Rat Model of Fragile X Syndrome

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Background: Atypical sound processing is one of the most common and quality-of-life affecting symptoms seen in autism spectrum disorders (ASD). Fragile X Syndrome (FXS) is the leading inherited cause of ASD and a majority of FXS individuals present with auditory processing issues. Despite this prevalence and centrality to the autistic phenotype, relatively little is known about the nature and mechanisms of auditory perceptual impairments in FXS and ASD.

Methods: We characterized auditory processing in adult male Fmr1 KO rats and wildtype littermates using a comprehensive battery of behavioral tasks that take advantage of the highly trainable nature of rats and their innate capacity to express complex behavior. We combined these behavioral assays with multi-region electrophysiological recordings and computational modeling to determine the neural origins of atypical sound-driven behavior in these animals.

Results: We found that Fmr1 KO rats exhibited heightened perceptual sensitivity and impaired gating of incoming auditory information, resulting in reduced sound tolerance and exaggerated avoidance behavior. Despite this behavioral evidence for sound hypersensitivity, Fmr1 KO rats exhibited diminished suprathreshold sound-evoked responses at the level of the auditory nerve and brainstem. However, this reduced afferent drive was progressively amplified along the ascending auditory pathway, culminating in several cortical network alterations that could account for observed perceptual differences, including increased spontaneous firing rates, steeper sound intensity-response functions, and broader frequency tuning. Using an experimentally informed Bayesian population decoder, we demonstrated that we could recapitulate behavioral deficits from auditory cortical but not subcortical neurophysiological recordings.

Conclusions: These findings are reminiscent of central gain changes observed along the central auditory pathway in response to hearing loss, suggesting a potential shared etiology for hyperacusis of diverse origin. Moreover, our results indicate that cortical hyperexcitability may account for a range of auditory behavioral phenotypes in FXS, providing a potential locus for development of novel biomarkers and treatment strategies that could extend to other forms of ASD.

SU12. Somatostatin Interneurons Drive Contrast Adaptation in the Mouse Primary Auditory Cortex

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Background: In natural environments, behaviorally-relevant sounds such as speech often co-occur with background noise such as wind or traffic. In order to effectively identify and respond to relevant sounds, the auditory system must isolate signal from noise. This ability is reduced in

people with hearing impairments and auditory processing disorders, posing unique challenges in navigating everyday environments. In order to develop effective treatments, it is vital to understand the mechanisms underlying hearing in noise.

One strategy for isolating signal from noise is to adapt to the current auditory environment. In the primary auditory cortex (A1), neurons adapt to changes in stimulus variability (contrast) by adjusting their gain. This process is known as contrast gain control (CGC). Though CGC has been demonstrated across many organisms from mice to humans, its mechanisms are still unknown.

In this study, I test the hypothesis that two different classes of inhibitory interneurons, parvalbumin (PV)- and somatostatin (SST)-positive interneurons, drive contrast gain control in mouse A1.

Methods: In order to test the role of different inhibitory interneuron subtypes in driving CGC, we optogenetically suppress PV or SST populations in A1 of mice presented with stimuli of changing contrasts. We test for CGC using two methods: behavioral performance on a target-detection task, and extracellular electrophysiological recordings in A1.

First, we tested how optogenetically suppressing different populations of interneurons affected behavioral performance on a target-detection task. Mice were presented with stimuli of low and high contrasts and trained to respond when they detect an increase in sound pressure level. Previous experiments in mice and humans show that performance on similar tasks increases in low-contrast backgrounds, corresponding to increased neuronal gain. If PV or SST neurons drive CGC, we expect optogenetic suppression of these populations to reduce the change in performance between the two contrasts.

Second, we performed acute extracellular recordings in mice passively exposed to auditory stimuli with high and low contrasts. Using these neuronal responses, we can directly calculate neuronal gain changes across stimulus contrasts. If PV or SST neurons drive CGC, we expect optogenetic suppression to reduce the degree of gain adaptation between the two contrasts.

Results: We find that SST suppression, but not PV suppression, impairs CGC between high and low contrast conditions. Though analysis of the extracellular data is still in progress, we observe a similar trend of decreased CGC during SST but not PV suppression. These results suggest that SST-positive interneurons drive CGC in mouse A1.

Conclusions: This research finds that SST neurons are involved in driving contrast adaptation in the cortex. This opens up new avenues of research into the potential role of SST neurons in auditory processing disorders.

SU13. Multiscale Adaptation Dynamics Reflect Hierarchical Predictive Processing in the Ferret Auditory Cortex

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Background: The mammalian brain encodes regularities with adaptation across multiple timescales: from rapid stimulus-specific adaptations in short-scale, to predictive processing of complex sequence structures. Previous studies, across species, suggest a cortical gradient of integration windows, from A1 to higher auditory and supramodal areas, but details of both their flow and temporal generalization remain unknown. Our goal is to dissociate responses to omission, local, and global violations in ferret A1 and PEG and to quantify their shared information and integration depth.

Methods: We recorded 32-channel electrocorticography (ECoG) covering the primary auditory cortex (A1) and the posterior ectosylvian gyrus (PEG) in ferrets, while presenting hierarchical sequences of pure tones or natural syllables. We used the local-global oddball structure as the main framework for our experiment design, and the sequences with various lengths comprised a repetition stage followed by a final event that either conformed to, violated, or was omitted in relation to the learned temporal pattern.

Across experiments, we manipulated: (i) final-item change after repetition (local violation), (ii) omission at sequence end, and (iii) higher-order pattern changes across different trials of the experiment run (global violation), including cross-item substitutions and re-ordering. Analyses combined condition-wise ERPs, time-resolved linear decoding, and temporal generalization matrices within and across areas. Finally, a dynamic Bayesian observer estimated per-channel integration depth by fitting trial-wise neural “surprise” derived from the event-locked mismatch windows.

Results: Local violations expressed an early MMN-like offset-locked response (< 200 ms) with strong shared information between A1 and PEG. Linear decoders indicated a stronger A1 contribution at this stage. A later component (≈ 250 – 500 ms) was more temporally sustained in PEG, showed broader temporal generalization within PEG compared to A1, and reduced cross-area decodability between A1 and PEG, representing area-specific updatings. Global violations emerged later (≈ 450 – 700 ms), partially dependent on the presence and magnitude of the earlier local response, and appeared as short sets of pulse-like signals in temporal generalization rather than sustained, time-invariant codes.

The Bayesian observer showed predictions consistent with the general pattern of our recorded surprisals. The model used to estimate the best-fitting integration depths and compare them in A1 and PEG. These effects are observable in tone sequences to syllabic sequences with different lengths and stimulus structures.

Conclusions: The results are consistent with hierarchical predictive processing with parallel mechanisms: fast, MMN-like, shared prediction errors tied to terminal-event changes are strongly expressed in both A1 and PEG, followed by longer-window, field-specific positive ERPs that are more prominent in PEG for the local deviation. We also observed meaningful responses for omission and global effects. Model-based estimations can investigate the scale of effective integration windows in each area. Together, these results quantify how cortical fields coordinate prediction errors and updates across timescales, linking computational prediction of surprise to mesoscale physiology.

SU14. Binaural Interaction Creates Diverse Azimuth Tuning Curve Shapes Across Auditory Midbrain Neurons

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Background: Neurons in the central nucleus of the inferior colliculus (ICC) exhibit spatial receptive fields due to underlying neural sensitivity to acoustic cues that covary with sound source location, including binaural cues (interaural time difference [ITD] and interaural level difference [ILD]), and monaural cues (spectral shape and acoustic gain within each ear). The present study examines how these cues shape each neuron's azimuth tuning curve (i.e., the function of firing rate vs. source location within the front horizontal plane), and whether the effects of cues differed with neural characteristic frequency (CF).

Methods: Broadband noise stimuli were presented to awake rabbits of both sexes in virtual acoustic space using each rabbit's own head-related transfer functions. Stimuli were presented either monaurally (contralateral ear) or binaurally, with transfer functions digitally manipulated in some conditions to isolate neural sensitivity to ITD or ILD.

Results: For low-CF neurons (< 2.8 kHz), azimuth tuning curves measured under monaural presentation were mostly flat, whereas curves measured under binaural presentation took on a diversity of shapes, such as functions sloping upwards or downwards from ipsilateral to contralateral azimuths; functions with broad, bell-shaped tuning to various azimuths; and functions with complex shapes. Binaural interaction (i.e., the effect on firing rate of two ears vs. contralateral ear only) caused facilitation of rates in most low-CF neurons; rate facilitation or suppression was not biased to particular azimuths and could be driven by neural sensitivity to ITD, ILD, or the covariation of both. Unlike low-CF neurons, high-CF neurons (> 2.8 kHz) had azimuth tuning curves measured under monaural presentation that took on a diversity of shapes; binaural interaction in these neurons changed the shapes of tuning curves, but the shapes remained diverse. Binaural interaction caused suppression of rates in most high-CF neurons, which was biased towards ipsilateral azimuths; however, rate facilitation found in a minority of neurons was not biased to particular azimuths. Unlike low-CF neurons, rate suppression or facilitation in high-CF neurons was overwhelmingly driven by neural sensitivity to ILD.

Conclusions: Consistent with a diversity of tuning curve shapes found across both low- and high-CF neurons, the distribution of firing rates over the neural sample evoked by the noise stimulus was constant across azimuths except for a small decrease at the most extreme ipsilateral locations (60°–90°). Therefore, the pattern of firing rate activity across ICC neurons was inconsistent with azimuth decoding models that rely upon summed population activity.

SU15. Aging and Synaptopathy Lead to Impaired Temporal and Binaural Processing in the Auditory Brainstem, and Spatial Hearing, in the Mongolian Gerbil

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Background: Aging is known to lead to difficulty in binaural and spatial hearing, often before affecting hearing thresholds. One potential source of this may be Cochlear Synaptopathy (CS), or the loss of synapses between inner hair cells and auditory nerve fibers. CS occurs during aging, and is present prior to raised hearing thresholds. The mechanisms of how age-related CS could disrupt binaural hearing are unclear, but they likely involve the auditory brainstem, the first site to receive input from the auditory nerve fibers and the earliest site for binaural processing. Here, we assess behavioral, physiological, and histological measures of auditory brainstem dysfunction from aging and synaptopathy in the Mongolian Gerbil (*Meriones unguiculatus*).

Methods: Cohorts consisted of young (2-10 month) and aged (> 30 month) gerbils. We recorded auditory brainstem responses (ABRs), envelope following responses (EFRs), spatial hearing behavior, and in vivo single-cell electrophysiological responses from cochlear nucleus and trapezoid body units. From the ABRs, we also measured the Binaural Interaction Component (BIC), a biomarker of spatial hearing abilities. To see if these measurements correlated with behavioral changes, gerbils were tested utilizing prepulse inhibition of the acoustic startle response (PPI) on two sets of tasks: 1) gap detection in broadband noise and 2) sound location spatial acuity. We also assessed synaptopathy by staining cochlea for presynaptic (Ctbp2) and postsynaptic (GluA2) markers and inner hair cell bodies (Myosin VIIa).

Results: We found an increase of synaptopathy, quantified by a decrease of colocalized pre- and postsynaptic markers per inner hair cell, in aged gerbils compared to the young cohort. This corresponded with a decrease in ABR Wave I and Wave III amplitudes, but not a significant reduction in hearing thresholds. The impaired ABRs and lack of threshold shifts indicate an impairment of neural synchrony in the brainstem. Aged animals exhibited reduced BIC amplitude, as well as impaired modulation of BIC amplitude with variation of interaural time differences (ITDs), indicating a reduction of brainstem binaural encoding in aged animals. We also found a reduced synchrony in EFR recordings, further showing physiological dysfunction in the auditory brainstems of the aged cohort. These findings corresponded with a reduction of synchrony to amplitude modulated stimuli recorded in vivo from bushy cell axons and medial nucleus of the trapezoid body (MNTB) neurons. Behaviorally, aged animals had higher thresholds of detection for both gap length and spatial acuity, indicating impaired temporal and spatial hearing behaviors.

Conclusions: Collectively, these data reveal that aged gerbils have impaired auditory brainstem physiology, partly as a result of synaptopathy, which underlies behavioral issues in temporal and spatial auditory processing similar to that exhibited by aged humans. [Supported by NIDCD 1F31DC021622-01]

SU16. Impaired Temporal Precision in Auditory Brainstem Processing Underlies Sound Localization Deficits in a Novel Mouse Model of Spinocerebellar Ataxia Type 13

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Background: Spinocerebellar Ataxia Type 13 (SCA13) is an inherited motor disorder characterized by an additional deficit in sound localization, despite otherwise normal hearing. This deficit arises from point mutations in the gene encoding the voltage-gated potassium channel Kv3.3, which is essential for neurons that support high-frequency firing, such as those in the auditory brainstem. To investigate the neural circuit mechanisms underlying this auditory impairment, we developed a novel CRISPR-Cas9 mouse model expressing the human R420H Kv3.3 mutation, which disrupts the S4 voltage sensor and impairs channel function.

Methods: The investigation was conducted using whole-cell patch-clamping, in vivo single unit recordings and imaging using immunostaining.

Results: Histological analysis of the auditory brainstem in R420H mutant mice revealed only a mild, age-unrelated reduction in cell numbers within the Lateral Superior Olive (LSO), without evidence of significant neurodegeneration. This suggests that the observed deficits are primarily functional rather than due to degenerative cell loss. A slight disruption in Kv3.3 protein transport was also noted, which might further contribute to the phenotype. However, the most pronounced effects of the mutation were observed in action potential (AP) dynamics.

Whole-cell patch-clamp recordings from brainstem slices demonstrated that the R420H mutation substantially broadens APs, particularly in the LSO (with a 2–3-fold increase in AP half-width) and to a lesser extent in the medial nucleus of the trapezoid body (MNTB; 1.5–2-fold increase). As a result, both MNTB and LSO neurons showed high failure rates during high-frequency stimulation (500 Hz in MNTB and 200 Hz in LSO). In the LSO, firing failures were strongly correlated with prolonged AP duration and were further exacerbated by significantly extended excitatory and inhibitory postsynaptic currents.

In vivo single-unit recordings confirmed that broadened APs reduce the maximum firing rates of MNTB and LSO neurons. Importantly, we found a marked imbalance between ipsilateral excitation and contralateral inhibition converging onto LSO principal cells. This disruption in synaptic integration manifested as a significantly reduced binaural interaction component (BIC) in auditory brainstem response (ABR) recordings.

Conclusions: Collectively, these results demonstrate that the R420H Kv3.3 mutation compromises the temporal fidelity of auditory brainstem neurons, disrupting the precise balance of excitation and inhibition necessary for accurate sound localization. This establishes a direct mechanistic link between ion channel dysfunction, neural circuit impairment, and the specific auditory deficits observed in SCA-13.

SU17. Active Sensorimotor Strategies for Sound Localization in Complex Acoustic Scenes

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Background: In natural environments, animals must react appropriately to behaviorally relevant sounds, such as those signaling predators or food sources, while ignoring competing auditory signals. Effective responses require identifying both the identity and location of sounds. The psychophysics of sound localization have been well characterized by presenting stimuli at varying positions relative to an animal's head, which is kept stationary for precise spatial control.

However, such approaches do not capture the sensorimotor strategies animals use during active navigation. In real-world settings, auditory spatial perception is shaped by environmental noise, behavioral context, and self-motion. Animals frequently orient their heads and bodies to reduce perceptual ambiguity and improve localization accuracy. To investigate the behavioral strategies that support naturalistic sound localization, we developed a freely-moving auditory discrimination task.

Methods: We trained C57BL/6J mice to localize a target sound along the perimeter of an open, circular arena while ignoring a distractor presented simultaneously from another azimuthal position. Stimuli were bandpassed white noise of varying center frequencies. After trial initiation, both stimuli played continuously until mice approached and licked a water spout beneath the perceived target. Since mice were free to explore before making a decision, this design enabled observation of how animals integrate dynamic sensory input with self-motion.

Results: Over several weeks of training, mice adopted characteristic sensorimotor strategies that facilitated task learning, including rapid head and body “scanning” movements at stimulus onset and 180° turns to correct errant approaches towards the distractor. These behaviors became more frequent with learning and coincided with increased accuracy, shorter reaction times, and more stereotyped trajectories.

Conclusions: These results demonstrate that in an unconstrained sound localization task, mice resolve spatial ambiguity with dynamic movement strategies and update motor plans continuously in response to ongoing acoustic input. Our ongoing work investigates the neural circuits underlying this dynamic sensorimotor loop. While auditory cortex encodes spectral features and learned associations, it lacks a map of egocentric space. In contrast, its projection targets in the midbrain, such as the superior and inferior colliculi, are spatially tuned and mediate orienting behaviors. We are currently using pharmacological and optogenetic perturbations to dissect how corticocollicular circuits contribute to spatial decision-making, offering new insight into how animals transform auditory input into adaptive action.

SU18. The Impact of Response Method on Sound Localization Performance

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Background: Sound localization is a key marker of binaural hearing, relying on the brain’s integration of interaural timing and level cues. Despite its clinical relevance, conventional localization assessments lack ecological validity and do not capture the reflexive nature of natural orienting. Standard methods, such as pointing or reporting a speaker number, require conscious decision-making and are susceptible to visual biases, potentially limiting their sensitivity to true auditory spatial perception. In natural listening, orienting to a sound involves a coordinated sequence of eye and head movements: the eyes typically move first, providing a rapid, low-latency estimate of target location, followed by slower head adjustments that align auditory and visual cues. We hypothesize that combined reflexive head-and-eye orienting offers a more direct and automatic measure of spatial hearing. This study examines how response modality influences localization performance in normal-hearing adults, with attention to both accuracy and precision.

Methods: Fifteen normal-hearing adults participated in a within-subject, repeated-measures design. Localization was assessed under two hearing conditions: normal binaural hearing and simulated monaural hearing (earplug + muff). Broadband Gaussian white noise (0.2–20 kHz) was presented at 50, 60, or 70 dBA from 19 loudspeakers spanning $\pm 90^\circ$ in azimuth, separated by 10° . Tasks were performed in both vision-allowed and vision-denied conditions to examine the influence of visual cues while eliminating feedback during trials. Participants completed three response modalities: (1) head movement alone, (2) combined head and eye tracking, and (3) verbal reporting of speaker number (vision only). For each trial, head and eye trajectories, reaction times, and response times were recorded. Localization performance was quantified using regression of target azimuth onto participant responses, providing gain, bias, and R^2 . Mean absolute error measured overall accuracy, while trial-to-trial variability captured precision.

Results: Response modality significantly influenced localization outcomes. Combined head-and-eye tracking demonstrated lower mean absolute error and reduced variability compared with head-only responses. Verbal reporting was associated with longer reaction times and higher variability, reflecting additional cognitive and motor demands. Simulated monaural hearing amplifies these differences, with reflexive orienting responses maintaining greater sensitivity to spatial cues than verbal responses. Regression analyses suggests improved alignment for combined head and eye tracking with true target locations.

Conclusions: Response method shapes both the accuracy and temporal dynamics of measured localization. These results suggest that reflexive orienting responses, particularly combined head and eye movements, provide a more precise and sensitive measure of auditory spatial perception than traditional methods. These findings underscore the importance of ecologically valid response modalities in both experimental and clinical assessments of spatial hearing. Careful consideration of response method has implications for evaluating auditory performance, designing experiments, and interpreting outcomes in hearing-impaired populations or users of hearing-assistive devices.

SU19. The Impact of Chloride Homeostasis on the Development of LSO Neurons

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Background: In the developing nervous system, inhibitory inputs are often transiently depolarizing due to high intracellular chloride concentrations, a process thought to guide neuronal maturation and synaptic refinement. In the auditory brainstem, this chloride gradient is primarily regulated by the transporters NKCC1 and KCC2. However, the specific role of this early depolarizing inhibition in shaping developing auditory circuits remains unclear.

Methods: To investigate the functional impact of early depolarizing inhibition, we used a mouse line with a double mutation in the KCC2 gene that enhances chloride extrusion and abolishes depolarizing GABA/glycinergic responses. We performed whole-cell patch-clamp recordings in acute brainstem slices targeting principal neurons of the lateral superior olive (LSO), a nucleus with well-characterized inhibitory inputs. Experiments were performed in animals at postnatal day 14 (P14) and P20, corresponding to the period shortly after hearing onset and following early auditory experience.

Results: We found that gross morphological development and intrinsic membrane properties of LSO neurons in KCC2 mutant mice were comparable to those in wildtype animals. However, afferent fiber stimulation revealed that amplitudes of inhibitory postsynaptic current (IPSC) amplitudes were significantly reduced with no change in IPSC kinetics or short-term plasticity. A similar reduction in current amplitude was observed with direct application of glycine, suggesting that the decrease in IPSC size is due to postsynaptic alterations such as a reduction of glycine receptors.

Conclusions: Our preliminary findings suggest that while early depolarizing inhibition is not essential for gross morphological development or maturation of intrinsic membrane properties of LSO neurons, it might be critical for fine-tuning synaptic strength, likely through a postsynaptic mechanism involving the regulating glycine receptor expression.

SU20. Maturation of Intrinsic Membrane Properties in Octopus Cells of the Mouse Cochlear Nucleus

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Background: During postnatal development, synaptic refinement and electrophysiological maturation occur simultaneously to finely tune auditory brainstem circuits. Before ear canal opening, spontaneous peripheral activity establishes precise circuitry across the central auditory system. When sound-driven activity begins, further refinement occurs through synaptic pruning. For octopus cells of the mammalian ventral cochlear nucleus, extreme intrinsic membrane properties are essential for the submillisecond temporal integration that supports spectral edge detection. Octopus cells in mature mice have low input resistances ($\sim 4\text{--}10\text{M}\Omega$), fast time constants ($\sim 200\mu\text{s}$), and large low-voltage-activated potassium and hyperpolarization-activated conductances ($\sim 40\text{nS}$ and $\sim 60\text{nS}$ at rest, respectively). These membrane properties, combined with the large diameter dendrites, allow for rapid and precise integration of auditory nerve input. We aim to define when these anatomical and physiological specializations emerge relative to the onset of hearing.

Methods: We examined the timeline of octopus cell maturation using in vitro whole-cell current clamp recordings from postnatal day 4 to 35 (P4–35) mice. Active and passive membrane properties including input resistance, time constant, HCN sag ratio, and action potential half-width were assessed. Auditory nerve inputs were evoked by electrical stimulation, and cells were filled with biocytin for post hoc three-dimensional reconstruction of dendritic arbors.

Results: By the time of ear canal opening (P10–12), intrinsic properties of octopus cells are relatively mature. Measures of input resistance, time constant, HCN sag ratio, and spike half-width are comparable at P10 and P16. Further, reconstructions of dendritic arbors revealed no meaningful changes between P10 and P16. Despite this stability at the onset of hearing, octopus cells underwent a rapid transformation between P4 and P10. During this early period, dominated by spontaneous cochlear activity, input resistance decreased, time constant shortened, sag ratio increased, and action potentials narrowed.

Conclusions: The timeline for anatomical and physiological development suggests that the properties underlying octopus cell function are established by the time sound-driven activity begins. Octopus cells rapidly mature when spontaneous waves of activity from the cochlea

dominate activity patterns in the octopus cell circuit. The rapid maturation that precedes ear canal opening highlights a critical developmental window shaped by spontaneous activity rather than acoustic experience. Octopus cells thus enter the onset of hearing configured for fast temporal integration. Future work will test how altering peripheral activity during this early window influences octopus cell development. By comparing normal and disrupted activity patterns, these experiments will define the contribution of spontaneous cochlear activity to the emergence of octopus cell function.

SU21. The Effect of Electrode Placement on the Auditory Brainstem Response

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Background: The auditory brainstem response (ABR) is commonly used both in the clinic and the lab to estimate hearing thresholds and examine the subcortical auditory system. However, the location of the neural generators and the size of the responses lead to poor signal-to-noise ratio (SNR), requiring long test times to acquire an adequate signal. Typically, earlobes or mastoids are used as the reference electrodes, but other reference locations may improve signal quality and speed response acquisition. Previous work has shown that the nape of the neck reduces muscle artifact in subcortically generated frequency-following responses, which may also benefit the ABR.

Methods: Subjects were presented with one hour of randomly timed click stimuli at 80 dB peSPL with a mean rate of 40 clicks per second. EEG was recorded using a Biosemi Active3 system with a 64-channel cap, and additional electrodes were placed on the earlobes, nose, nape of the neck, and collarbone for offline re-referencing during analysis. Two more electrodes were placed by the left eye to aid in the removal of eye-related artifacts.

Responses were computed separately for the left ear and right ear clicks. The SNRs of the responses were estimated based on the variance of the waveform in the response window compared to the baseline variance. For each reference electrode, responses were re-referenced and the SNR was calculated for the response from each electrode in the cap to identify optimal placement of electrodes to obtain the ABR.

Results: Preliminary results indicate the nape and earlobes to be the best choices for reference electrodes, producing responses with the highest SNRs. The nape provides a larger ABR wave V and less muscle artifact than earlobes. The combined earlobes had the next best SNR. Wave I was most visible when referenced against the ipsilateral earlobe. The positive electrodes with the best SNRs were located near the vertex of the head for all references, with commonly used frontal electrodes showing worse SNR.

Conclusions: The specific electrode montage can have a large impact on the SNR of ABRs. In a bipolar montage, the location of both electrodes should be considered in the context of the specific goals of the recording (e.g. fast overall response detection versus examination of wave I). Selecting the optimal montage may yield substantial improvements in response quality, which is especially important when recording time is limited.

SU22. Dopamine Receptor Expression in the Mouse Superior Olivary Complex

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Background: Neuromodulatory circuits influence auditory neural computations by regulating presynaptic and/or postsynaptic mechanisms at multiple levels of processing. However, modulation in auditory regions between the periphery and the cortex have remained largely unexplored. Recent work by our laboratory has demonstrated several mechanisms of modulation in the superior olivary complex (SOC) by a network of cholinergic inputs (Beebe et al. 2021, Zhang and Burger 2024, Weimann et al. 2024). In addition to cholinergic input, dopaminergic projections to the SOC have been documented (Nevue et al. 2016). However, there have been no studies demonstrating expression of dopaminergic receptors or physiological modulation by dopamine in SOC neurons. While dopaminergic neurons compose a small population in the brain, they have potent and widespread impacts on targets throughout the nervous system. A diverse array dopamine receptor subtypes (D1-5), underlie the variable physiological outcomes of dopaminergic modulation. Dopamine receptors are members of the G protein-coupled receptor family and divided into two classes. D1 and D5 belong to the D1-like family while D2, 3, and 4 are members of the D2-like family. Each of these families of dopamine receptor subtypes initiate unique downstream cascades.

Methods: To investigate the potential auditory processing function(s) of the dopaminergic input to the SOC, we first used immunohistochemistry to describe the expression patterns of dopamine receptor subtypes 1-5 in mouse SOC neurons in mice aged P10 and P30 before and after hearing onset (~P12). Antibodies directed against D1-D5 receptors were used to examine expression in the principal nuclei of the SOC. Presynaptic expression was confirmed by colocalization of dopamine receptor stain with VGLUT1.

Results: We demonstrate that all five DA receptor subtypes are differentially expressed in the mouse SOC. D1 is expressed in SOC nuclei pre and post hearing onset. In the MNTB, D1 appears to have weak expression early in development with strong post-synaptic expression later at P30. D2 appears to be both pre and postsynaptically expressed at P10 with primarily postsynaptic expression by P30. D3 demonstrates a primarily presynaptic expression pattern across development in the MNTB and the LSO. D4 exhibits presynaptic expression at P10 with stronger postsynaptic expression by P30 in the MNTB while the LSO exhibits primarily postsynaptic expression. Finally, our staining suggests that D5 receptors are weakly expressed across development in SOC nuclei.

Conclusions: Our results show for the first time that DA receptor subtypes 1-5 are expressed in the mouse SOC with unique expression profiles. These results strongly suggest that dopamine is likely to provide neuromodulatory signaling to the SOC.

SU23. Age-Dependent Regulation of Calcium Responses in the Mouse Medial Nucleus of the Trapezoid Body Neurons

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Background: In the central auditory system, Ca²⁺ signaling plays a pivotal role in the regulation of cellular processes including neuronal development and maturation. Disruption of Ca²⁺ homeostasis and signaling causes abnormal development of auditory circuits, compromising auditory processing and behavior. Here, using Ca²⁺ imaging in brain slices obtained from mice that genetically expressed calcium indicator GCaMP in glycinergic cells, we investigated the underlying mechanisms for developmental Ca²⁺ regulation in neurons of the medial nucleus of the trapezoid body (MNTB), a brainstem nucleus involved in sound localization.

Methods: Brainstem slices with visually identified MNTB were prepared from GlyT2-Cre/tdT-GCaMP6f mice of either sex, which is a transgenic line obtained by crossing the glycine transporter 2-Cre recombinase mouse to the reporter line ROSA26-tdT-GCaMP6f. Ca²⁺ imaging experiments were performed at several milestone ages of development including P0, P7, P14 and P21, at 35 °C. Fluorescence images of principal MNTB neurons were obtained under excitation wavelength of 480 nm. Ca²⁺ signals were recorded in response to activation of various excitatory and inhibitory transmitter receptors through bath application of their respective agonists and under electrical stimulation applied to their afferent inputs.

Results: Prior to hearing onset at postnatal day 7 (P7), Ca²⁺ signals were produced in MNTB neurons when the glutamate receptors (NMDARs, AMPARs, group I mGluRs) were activated by bath application of their respective agonists (NMDA 200 μM, AMPA 100 μM, 3,5-DHPG 200 μM). After hearing onset (P14 and P21), the responses declined dramatically for NMDA receptors and group I mGluRs. AMPAR-mediated signals remained relatively high. In contrast, Ca²⁺ transients in response to GABA (100 μM) and glycine (200 μM) were only observed in neonatal mice and diminished a few days after birth. Transmitter receptor-mediated Ca²⁺ signaling was largely blocked by their specific antagonists. Furthermore, electrical stimulations applied to the excitatory afferent fibers innervating MNTB, triggered release of glutamate which elicited Ca²⁺ signals, in a stimulus intensity- and frequency-dependent manner, via glutamate receptors and potentially voltage-gated Ca²⁺ channels. Although, cells from all experimental age groups were able to generate action potentials with no significant differences, under whole cell patch-clamp recordings, using similar stimulation parameters. However, calcium signals elicited in response to electrical stimulations, followed similar down-regulation fashion as the drug applications, in the MNTB neurons, over the ages of development.

Conclusions: Taken together, Ca²⁺ signaling in MNTB neurons, mediated by various transmitter receptors was developmentally down-regulated and subject to refinement by hearing experience.

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SU24. Effects of Cochlear Synaptopathy on Single-Unit Cochlear Nucleus Responses in the Budgerigar

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Background: Cochlear synaptopathy (CS) is a commonly occurring pathology in aging humans, defined as the loss of synapses between hair cells and auditory-nerve fibers (ANFs). CS is hypothesized to impair perception of sounds in noise, but underlying neural mechanisms are unknown. Recent studies suggest that CS can amplify auditory-nerve onset responses and alter temporal dynamics. However, effects of CS on brainstem encoding remain less explored. The current study quantified effects of CS on neural encoding in the nucleus angularis (NA) of the budgerigar, a parakeet species. NA is one of two avian brainstem cochlear nuclei receiving direct input from the auditory nerve. Budgerigars were selected based on recent studies of kainate-induced CS in this species and previous behavioral research demonstrating performance comparable to humans for various complex auditory discrimination tasks.

Methods: The current experiment compared basic NA response properties including frequency tuning curves, post-stimulus time histograms (PSTHs), and rate-level functions between control and CS-induced budgerigars. In CS animals, substantial (60-70%) ANF loss was induced via intracochlear infusions of 1-2 mM kainic acid. Distortion-product otoacoustic emissions were used to confirm normal hair-cell function. For in-vivo recordings of NA units, a craniotomy was made above the anterior, vertical semicircular canal. A glass electrode with 40 to 80 M Ω impedance was placed in the flocculus of the cerebellum and driven into NA to record single-unit responses. PSTHs were obtained with a characteristic-frequency (CF) tone presented 30 dB above threshold. Rate-level functions were created by randomly presenting CF tones of varying sound levels (0 to 80 dB SPL).

Results: NA neurons in control budgerigars had primary-like, onset, and chopping PSTHs in response to CF tones. Tuning curves were V-shaped, with characteristic frequencies within the range of the budgerigar's behavioral sensitivity (i.e., up to 5-6 kHz). Strength of synchrony was calculated from PSTHs using vector strength (VS). VS was greatest below 1 kHz and generally insignificant above 2 kHz, in contrast to budgerigar ANFs which show significant VS up to 4-5 kHz. From rate-level functions, we found that some NA units monotonically increased firing rate as sound levels increase, while others had nonmonotonic rate-level functions. NA units in CS-induced animals showed the same PSTH response categories and had broadly similar tuning curves, VS of phase-locking, and rate-level functions to control units. Population results are compared between groups to quantify changes in NA encoding with CS.

Conclusions: Preliminary results suggest that NA units in the CS-induced budgerigars have similar characteristics to control units. Further analyses will test population differences and characterize potential changes in onset activity, adaptation, and envelope encoding in CS-induced animals. Research in this species can provide important insight into the neural bases of normal and hidden hearing loss.

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SU25. Semilunar Nucleus: Physiology, Connections, and mGluR Modulation

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Background: Besides the calyx of Held input, additional non-calyceal inputs to the medial nucleus of the trapezoid body (MNTB) have been reported, but their functions and origins are largely unknown. Recently, a non-calyceal input originating from the semilunar nucleus (SLN) to the ipsilateral MNTB was identified in the rat (Gómez-Martínez et al. 2023, Front Neuroanat). To understand the function of this novel neural circuit, we investigated the physiology and connectivity of SLN in mice, with a focus on neuromodulation mediated by group I metabotropic glutamate receptors (mGluR1 and mGluR5).

Methods: We used a combination of brain slice electrophysiology, immunohistochemistry, and viral tracing. We conducted whole-cell patch clamp and cell-attached recordings at 35 °C in brainstem slices from mice of two age groups (< HO, before hearing onset, P7-P12; > HO, after hearing onset P13-P39). The SLN was identified by anatomical landmarks and/or calbindin1 expression pattern. Immunohistochemistry was used to examine the expression of mGluR1, mGluR5, and calbindin1. Retrograde viral tracing was used to reveal connections between SLN and other auditory structures. Mean and number of cells are reported.

Results: SLN neurons were highly excitable, with a low current threshold (< HO, 47.7 pA, n=60; > HO, 35.0 pA, n=12), partly owing to their high input resistance (< HO, 406.7 MΩ, n=56; > HO, 452.0 MΩ, n=13). A subpopulation of SLN neurons (< HO, 8/38 cells; > HO, 1/7 cells) exhibited spontaneous spikes at rest. Activation of group I mGluRs with 3,5-DHPG (200 μM) enhanced the excitability of SLN neurons and increased their spontaneous spike rate (< HO, control, 0.71 Hz, n=37; DHPG: 4.42 Hz, n=37; paired t-test p < 0.0001; > HO, control, 0.20 Hz, n=7; DHPG: 7.24 Hz, n=7; paired t-test p = 0.0156). The enhancement was partially blocked by an antagonist for mGluR5 (MPEP, 1 μM). The modulatory effect seemed long-lasting before and shorter after hearing onset. Electrical stimulation of the area dorsal to the SLN evoked EPSCs in SLN neurons, which summated temporally to 100 Hz synaptic stimulation. Ipsilateral electrical stimulation putatively activating the input from SLN to MNTB evoked glutamatergic EPSCs in MNTB neurons. Triple immunostaining confirmed the presence of mGluR1 and mGluR5 within the SLN, revealing a heterogeneous distribution pattern surrounding calbindin1-immunoreactive neurons. Retrograde viral tracing confirmed a descending projection of SLN neurons to the ipsilateral MNTB in mice. The MNTB-projecting SLN neurons in turn receive descending projections from the inferior colliculus and auditory cortex.

Conclusions: Neurons in the mouse SLN were intrinsically highly excitable and received a glutamatergic input via a descending pathway. Neurons in the MNTB received a non-calyceal glutamatergic projection originating from the ipsilateral side. Group I mGluRs enhanced the SLN excitatory output, potentially forming a tonic excitation in MNTB neurons, which may improve their temporal processing.

SU26. Why Some Listeners Shine in the Cocktail Party: Brainstem Envelope Coding Explains It

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Background: Understanding speech in complex acoustic environments is a fundamental challenge of everyday listening. Even among individuals with clinically normal hearing, there is

wide variability in the ability to follow a target voice amidst competing talkers. This “cocktail party problem” arises from both energetic masking (overlapping sound energy) and informational masking (confusion between similar voices), yet the neural mechanisms underlying individual differences remain poorly understood. One candidate mechanism is the precision of brainstem encoding of temporal envelope periodicity, which supports the extraction of pitch cues and voice segregation. While amplitude-modulated brainstem responses reliably reflect phase-locking to periodicity, it is unknown whether individual differences in suprathreshold envelope coding predict behavioral benefits in speech-in-speech tasks versus speech in steady-state noise. We hypothesized that individual differences in suprathreshold periodicity encoding, particularly at reduced modulation depths, would correlate with behavioral measures of talker-sex release from masking, but not necessarily with performance in steady-state noise.

Methods: we measured ASSRs at 101 Hz to amplitude-modulated stimuli at 40% and 100% AM depths in a cohort of normal-hearing adults ($n=30$; mean age = 20.73 years; females=28). Neural responses were quantified using phase-locking value (PLV) obtained from Wavelet transform and signal-to-noise ratio (SNR). Behavioral performance was assessed using a two-talker speech-in-speech task, designed to elicit talker-sex release from masking, and a digit triplet in speech-shaped noise.

Results: Revealed a selective relationship: PLV at 40% AM robustly predicted the magnitude of talker-sex release from masking ($r \approx 0.35$, $p = 0.015$), whereas PLV at 100% AM and SNR measures did not. No significant associations were observed between any neural metrics and speech-in-noise performance. These findings indicate that suprathreshold envelope encoding in the brainstem captures individual differences in neural fidelity that are critical for voice segregation, but not for overcoming energetic masking.

Conclusions: Our data support the idea that precision of suprathreshold temporal encoding at the brainstem level underlies listeners’ ability to exploit pitch differences between competing talkers. The absence of a relationship with speech in speech-shaped noise performance underscores the distinct neural mechanisms for informational versus energetic masking. These findings highlight the importance of assessing suprathreshold envelope encoding when predicting speech-in-speech perception and may inform future strategies for assessment and intervention for speech-in-noise deficits in listeners with normal audiograms.

SU27. Spatial Gradient of Synaptic Input Subtypes on Bushy Cells Along the Lateral- Medial Axis of the Anteroventral Cochlear Nucleus

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Background: Bushy cells in the anteroventral cochlear nucleus (AVCN) are among the first central auditory neurons to receive input from the auditory nerve (AN). These principal cells are innervated by large, specialized synapses, known as the endbulbs of Held, from spiral ganglion neurons (SGNs) that enable precise transmission of temporal fine structure in sound. Our previous study showed that bushy neurons are innervated by various combinations of AN synapses from different subtypes of type-I SGNs. However, it remains unclear whether any spatial distribution pattern exists among different subtypes of AN synapses in AVCN bushy

neurons. In this study, we investigated whether bushy cells are spatially organized along the lateral-medial axis of the cochlear nucleus based on the distribution of different AN input subtypes.

Methods: To investigate this spatial organization, we performed immunohistochemical labeling in the cochlear nuclei of CBA/CaJ mice. Cochlear nuclei were dissected out, cryo-sectioned, and stained using antibodies against calretinin, vGluT1 and vGluT2 to probe the distribution of different AN input subtypes on bushy cells.

Results: We identified gradients of AN input subtypes along the lateral-medial axis of the AVCN. Calretinin-positive type Ia synapses were more densely expressed on the lateral side, whereas vGluT2-positive non-type Ia synapses showed higher expression medially. This spatial distribution suggests that bushy cells receive input from distinct AN subtypes depending on their position within the AVCN.

Conclusions: Our findings reveal a spatial gradient of synaptic input along the lateral- medial axis on bushy cells in the AVCN. It suggests that in addition to the tonotopic organization along the dorsal-ventral axis and the differential cell-type distribution along the rostral-caudal axis, the AVCN may also be functionally compartmentalized along the lateral-medial axis. This spatial differentiation likely contributes to the processing of complex auditory information from diverse AN inputs.

SU28. Expression of Kv4 Channels in Bushy Neurons of the Cochlear Nucleus: An Immunohistochemical Study

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Background: Bushy neurons housed in the anteroventral cochlear nucleus (AVCN) are specialized neurons that process temporal sound information critical for auditory function. These neurons possess unique biophysical membrane properties due to the expression of various ion channels. These include voltage-gated potassium channels. Our previous study used brain slice electrophysiology to isolate A-type currents mediated by Kv4 channels in most bushy neurons and revealed their role in regulating neuronal excitability and temporal precision of spikes. However, conflicting observations were reported by multiple studies, in which no A-type current was found in bushy neurons using electrophysiology. To further confirm our previous findings and characterize expression patterns of Kv4 channels in bushy neurons, we investigated the expression of Kv4.2 subunit channels in AVCN bushy neurons, using immunohistochemistry.

Methods: We investigated Kv4.2 expression in bushy cell through immunohistochemical staining and confocal microscopy in CBA/CaJ mice. Antibodies against vGluT1 and Kv4.2 subunit were utilized to identify bushy neurons and investigate their expression of Kv4 channels. Specifically, bushy neurons were identified through the innervation of end bulbs of held synapses around cell bodies labeled by vGluT1. Cell bodies were corroborated through DAPI staining. Bushy cell expression of Kv4 channels was qualitatively analyzed through Kv4.2 presence on cell bodies.

Results: Immunohistochemical staining showed that most bushy neurons of the AVCN were labeled by Kv4.2 antibody. No colocalization between vGluT1 and Kv4.2 staining was observed, suggesting that end bulb of Held synapses do not express Kv4 channels.

Conclusions: These results confirmed our previous findings that a majority of bushy neurons express Kv4 channels. These channels help shape the biophysical membrane properties and promote temporal precision of spikes in these neurons. Our study suggests that A-type currents play an important role in processing auditory information at the first neural station of the central auditory system. This is expected to impact higher auditory nuclei and promote hearing sensation.

SU29. Diffusion Tensor Imaging of Brainstem Auditory Pathway in Controls and Prospective Cochlear Implant Patients

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Background: Cochlear implants are implantable devices capable of partially restoring hearing via electrical stimulation of the auditory nerve, mimicking normal hearing conditions. Outcomes following implantation vary widely, particularly among patients with long durations of deafness. This variation limits clinicians' ability to predict who will benefit from the procedure. Neurobiological changes in the auditory pathway due to prolonged auditory deprivation may cause this variability, however, current objective markers are poorly characterized. Diffusion tensor imaging, a magnetic resonance imaging technique sensitive to white matter microstructure, offers a noninvasive means to assess subcortical auditory pathways. Some studies have reported a link between categories of auditory performance scores, but there is currently no set of biomarkers for candidate screening.

Methods: Data acquired from 23 participants were used in this project: 15 adult cochlear implantation candidates (aged 39-77) with sensorineural hearing loss, and eight high-school aged male students without reported hearing deficits as a reference group. MR imaging involved both T1-weighted and diffusion-tensor weighted scans (Patients: Philips MR7700, 1.7mm isotropic resolution; Controls: GEHC MR750, 2mm isotropic resolution).

T1 scans were registered to MNI152 space using FLIRT and FNIRT, and diffusion-weighted scans were processed using DiPy and FSL. Procedures included LPCA denoising, Gibbs ringing correction, brain extraction, and distortion correction via TOPUP and EDDY functions. White matter partial volume maps were generated via FAST and registered to DTI space. Tract masks were generated by using regions of interest (ROIs) associated with key auditory brainstem nuclei, such as the superior olivary complex (SOC), inferior colliculus (IC), and medial geniculate body (MGB) as key landmarks.

Average MNI space masks were obtained from Brainstem Navigator, registered using DiPy into both T1 and DTI space for all subjects, and averaged within population to create landmarks for

tract mapping. Probabilistic tractography was used on a subject specific basis and fit to the Constrained Spherical Deconvolution model, individually, for the left and right hemispheres. Streamlines that passed through all three ROIs were preserved.

A set of ROIs was generated from each population (Control and Patient) for the SOC, IC, and MGB. Group-specific ROIs were generated to account for variable partial volume averaging in the T1-weighted scans, ensuring preserved tractography would reside in the tissue.

Results: White matter bundle similarity was compared between the Patient and Control populations using DiPy's Bundle Analysis (BuAn) framework. No statistically significant differences were observed for these groups, but a modest linear relationship (r -squared = 0.45) was observed between the bundle similarity measure and age in the Patient population.

Conclusions: A small-sample pilot suggests a potential relationship between brainstem white matter fiber tract integrity and size and age of a prospective cochlear implantee. Further data collection is ongoing to expand the sample size and develop more uniform anatomical targets for group comparisons.

SU30. Detecting Individual Auditory Brainstem Responses (ABRs)

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Background: The Auditory Brainstem Response (ABR) is a measure of neural activity in response to sound stimuli. It is widely used for diagnosing hearing loss in humans and other animals. Current practice estimates hearing thresholds by identifying peaks in the mean ABR over hundreds of trials. However, thresholds based on the mean ABR alone decrease without bound as the number of trials used to estimate the mean increases.

We propose to score the similarity of each ABR trial with the population of ABR trials using covariance. By comparing the score distributions of stimulus-present and stimulus-absent trials, we define an ABR threshold based on the discriminability index d' . We show this measure converges as the number of trials increases.

Methods: We build a model to assess our new method, compare our new method with conventional methods, and confirm the theoretical basis for our method with a simple model and simulations.

We simulate ABR trials by generating deterministic waveforms with added Gaussian white noise. Increasing the amplitude of the deterministic waveform mimics responses to sounds of increasing stimulus level.

We then filter the simulated trials and calculate their mean for each stimulus level. At a given level, each individual trial is assigned a score equal to its covariance with the mean. The score distribution quantifies similarity between the trials.

Lastly, we compute the discriminability index d' value between the score distributions of a stimulus-present condition (non-zero waveform amplitude) with that of a stimulus-absent condition (no deterministic waveform). Because d' is the distance between the distributions, a stimulus is detected when d' is sufficiently large (for example, $d' > 1$). The stimulus level at which stimulus is detected is defined as the ABR threshold.

Results: We show that d' converges to a constant value as the number of trials increases. Consequently, ABR thresholds based on d' no longer decrease without bound as the number of trials increases. Using our simple model, we show how d' is related to the signal-to-noise ratio.

Conclusions: Previous ABR methods use the mean ABR waveform alone, causing the ABR threshold to depend on the number of stimulus trials. In this study, we introduce an alternative approach that uses covariance and d' to detect ABRs. This method defines an ABR threshold that becomes independent of the number of trials as the number increases. Future work will assess the utility of this approach using human and animal ABR data.

SU31. Enkephalin Regulation of Cochlear Nucleus Neuronal Function After Noise Exposure

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Background: While molecular and functional changes in inner ear cells post-noise exposure have received extensive attention, auditory brainstem alterations remain poorly understood. The cochlear nucleus receives signals from cochlear spiral ganglion neurons, processes/integrates them, and transmits to higher auditory centers. We show that single intense noise exposure induces pronounced molecular and functional changes in the cochlear nucleus as early as 1 day post-exposure, enriched in processes including synaptic transmission, ion channel activity, and cytoskeletal regulation. Notably, these changes persist to at least 3 days post-exposure. Concurrently, we found enkephalin plays an intriguing role here and investigated its potential cochlear nucleus protective function post-noise exposure.

Methods: We exposed 7-8-week-old C57BL/6J mice to 8-16 kHz noise at 109 dB SPL for 2 hours. The mice were then euthanized and dissected at 24 hours and 72 hours post-exposure to harvest cochlear nuclei, which were used for transcriptome sequencing or other molecular biology experiments. The results of RNA sequencing (RNA-seq) were validated using quantitative PCR (qPCR) or Western blotting (WB). Additional techniques employed included immunofluorescence staining, in situ hybridization, anterograde monosynaptic viral tracing, tissue clearing, whole-cell patch clamp recording, and auditory brainstem response (ABR) testing.

Results: Profound alterations in cochlear nucleus gene expression were observed at 1 day post-noise exposure. Enrichment analysis revealed the top enriched biological processes as synaptic reorganization, transsynaptic signaling, ion channel function, and cytoskeletal remodeling. Notably, proenkephalin (Penk) was the sole neuropeptide upregulated post-exposure, with sustained elevation through 3 days. Whole-cell patch clamp recordings localized Penk expression primarily to vertical cells of the dorsal cochlear nucleus (DCN). Via mWGA anterograde monosynaptic viral tracing, we demonstrated that Penk⁺ vertical cells innervate bushy and stellate cells in the ventral cochlear nucleus (VCN), which co-express δ - and μ -opioid receptors.

In vitro enkephalin perfusion induced hyperpolarization of resting membrane potential and reduced action potential firing in VCN neurons, suggesting a potential protective role against noise-induced hyperexcitability. Pretreatment with the δ -opioid receptor agonist SNC80 prior to noise exposure significantly ameliorated ABR threshold elevations, along with improved wave II latency and amplitude at 3 days post-exposure. Transcriptome sequencing and WB validation further revealed that δ -opioid receptor activation exerts neuroprotective effects—including anti-inflammatory and anti-apoptotic actions—via pathways including PI3K-AKT, MAPK, and NF- κ B.

Conclusions: A single intense noise exposure induces profound changes in gene expression in the mouse cochlear nucleus and upregulates proenkephalin (Penk) expression in vertical cells of the DCN. This upregulated Penk, via axonal innervation, modulates the electrophysiological properties of neurons in the VCN that express enkephalin receptors, including δ - and μ -opioid receptors. Prior to noise exposure, intraperitoneal administration of the δ -opioid receptor agonist SNC80 exerts neuroprotective effects in the cochlear nucleus—including anti-inflammatory and anti-apoptotic actions—and improves ABR metrics at 3 days post-exposure.

SU32. Triple AAV Vectors Enable Efficient and Safe Cochlear Gene Delivery for Large Genes in Adult Mice

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Background: Several reports have demonstrated auditory restoration after intra-cochlear gene therapy in patients with OTOF-related hearing loss. However, as nearly 200 causative genes have been identified in hereditary deafness, expanding the scope of target genes for cochlear gene therapy is necessary. Most studies of hereditary hearing loss employ adeno-associated virus (AAV) vectors, but their carrying capacity is limited to ~4.7 kb. Because some deafness genes exceed this size, packaging into a single AAV capsid is unfeasible, and splitting the transgene into two or three parts may be required. While successful dual-AAV-vector approaches have been reported, no study has yet demonstrated the feasibility of triple-AAV delivery. We therefore aimed to establish a triple-AAV cochlear delivery strategy that achieves apex-to-base transduction with high efficiency and minimal cochlear damage.

Methods: Enhanced green fluorescent protein (EGFP) was divided into three parts and packaged separately into AAV2/2 vectors (each titer $> 10^{13}$ GC/ml; VectorBuilder, construct patent pending). C3H/HeNjcl mice (5–6 weeks old) were used. In the left ear, 1 μ l of the triple AAV mixture was co-injected into the cochlea using the round window membrane + canal fenestration (RWM+CF) method we previously described. Auditory thresholds were assessed by auditory brainstem response two weeks post-injection. Cochleae and vestibular organs were then harvested and dissected to evaluate transduction efficiency.

Results: Robust transduction (40–50%) was observed in inner hair cells (IHCs) across all cochlear turns (apex, middle, and base). In contrast, outer hair cells (OHCs) and vestibular hair cells (utricle, saccule, semicircular canals) were not transduced. Importantly, auditory thresholds did not differ between injected and uninjected ears, indicating that the procedure did not affect hearing.

Conclusions: This study is the first to demonstrate that cochlear gene delivery using triple-AAV vectors is both efficient and safe for transducing IHCs in the adult murine cochlea. These findings suggest that triple-AAV strategies could be broadly applicable to the development of gene therapies for hereditary hearing loss caused by large deafness genes, such as CDH23, MYO15A, and USH2A that exceed the packaging limit of a single or dual AAV vector.

SU33. GJB2 Gene Therapy-Response of Two Pre-Clinical Mouse Models of the Most Frequent Form of Human Deafness, DFNB1

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Background: More than 1.5 billion people worldwide live with hearing loss (HL). Sensorineural HL affects one new-born in 700-1000 and approximately one child or young adult in 500 before the age of 20. Approximately 70% of congenital severe to profound deafness cases are hereditary due to monogenic defects, thus potentially treatable by gene therapy (GT).

GJB2-related autosomal recessive non syndromic HL (also referred as DFNB1) is the most common genetic cause of congenital sensorineural HL in many human populations, frequently accounting up to half cases. In addition, it is also responsible for late onset deafness, occurring even after 40 years of age. GJB2 encodes gap-junction channel protein connexin-26 (Cx26), involved in metabolites and ions homeostasis in the cochlea, necessary for cochlear development and sensory hair cell function and survival. Notably, Cx26 contributes to the endocochlear potential, the driving force of the sensory mechanotransduction. Hundreds of pathogenic GJB2 variants including large deletions, loss-of-function, and missense variants have been reported which outcomes spanning from mild to profound deafness.

Methods: To develop GT approaches for deafness caused by Gjb2 defects, we generate two conditional knockouts mouse models (as Gjb2 inactivation results in embryonic mouse lethality) crossing transgenic Gjb2loxP mice with Cre mice which display strong and wide Cre recombinase cochlear expression (i.e. in almost all Gjb2-positive cells). Both models display profound congenital deafness, severe cochlear architecture abnormalities and reduced endocochlear potential

Results: ear delivery of a GT-Gjb2 recombinant adeno-associated virus (AAV) results in a strong and long lasting (at least 6 months) Cx26 expression in most of Cx26 expressing cells of the cochlea especially in Hensen's cells, Deiters' cells, inner and outer pillar cells, inner phalangeal cells, inner border cells and inner sulcus. Importantly, no Cx26 was detected in hair cells in any of the 2 models. In both models, GT-Gjb2 improves ABR threshold up to 3 months post administration and preserve cochlear structure in almost 90% of mice. Furthermore, GT-GJB2 strongly reduce hair cells degeneration and neurons loss.

Conclusions: Thus, GT-GJB2 improves audition in these preclinical models of Gjb2 defects

SU34. High-Throughput Screening Uncovers the Hair Cell-Specific Promoter for Gene Therapy

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Background: Hearing loss is the most common sensory disorder globally, affecting over 400 million people, including 34 million children. Approximately 1 in 500 newborns suffer from hearing impairment, with genetic factors accounting for about 60% of cases. Adeno-associated virus (AAV)-mediated gene therapy has emerged as a promising treatment strategy for genetic hearing loss. However, the complexity of the cochlea—particularly its diverse cell types such as hair cells, supporting cells, and spiral ganglion neurons—poses significant challenges to precise gene delivery targeting. This study focuses on the systematic screening and identification of specific promoters for inner ear hair cells. By combining AAV vectors with high-throughput promoter screening, we achieved targeted delivery and precise expression in different types of cochlear hair cells, effectively overcoming previous issues such as off-target effects, low safety, and high dosage requirements in viral delivery. This work provides critical technical support for the clinical translation of genetic therapies for hereditary hearing loss.

Methods: Utilizing cochlear single-cell sequencing data, we conducted bioinformatic analysis and molecular cloning to construct a diverse hair cell promoter library encompassing various subtypes. A two-phase screening strategy was employed: high-throughput primary screening with a fluorescent reporter system identified candidate promoters, followed by precise secondary evaluation of their strength and specificity. An AAV-based gene therapy system was microinjected into Pjvk^{-/-} mice inner ears. Auditory function was assessed using ABR and DPOAE measurements, while immunohistochemistry evaluated protein expression and cell survival to determine system efficacy and safety.

Results: Through systematic screening, specific promoters for different types of hair cells were identified, enabling highly efficient and specific EGFP expression in hair cells. Based on one of these hair cell-specific promoters, HCpro, an AAV-mediated gene therapy system targeting hair cell-related deafness gene was designed. This system was delivered via microinjection into the inner ear of Pjvk^{-/-} mice. Auditory function was evaluated at multiple points post-treatment, revealing significantly reduced response thresholds compared to the untreated control group. Furthermore, no obvious adverse effects were observed in the cochleae of the treated mice, demonstrating the favorable safety and efficacy of this AAV-based gene therapy system. Ultimately, the treatment successfully restored auditory function in this genetic model of hearing loss.

Conclusions: Distinct from conventional promoter screening approaches, this study established an efficient and systematic strategy for identifying hair cell-specific promoters and successfully identified HCpro. This work not only provides a novel promoter option for the design of gene therapies targeting hair cell-related deafness genes but also opens avenues for extending

treatment strategies to other cochlear cell types, such as supporting cells and spiral ganglion neurons.

SU35. A Directed Evolution Novel Aav Mediated Gene Therapy Restores Hearing in Otof Mutant Mice and Patients

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Background: OTOF commonly causes non-syndromic auditory neuropathy, that affects 2.3-10% of patients with hereditary hearing impairment. OTOF only expresses in inner hair cells (IHC), crucial in synaptic vesicle cycling. Dual AAV mediated gene therapy has successfully restored hearing in patients with OTOF mutations. AAV is a single-stranded linear DNA defective virus with multiple serotypes targeting different tissues or cells. IHC-specific AAV vector still remains a major challenge.

Methods: In this study, we developed a platform to engineer novel AAV vectors through capsid mutation, peptide insertion, DNA shuffling, and promoter modification. A novel AAV vector, AAV-WM04, was engineered, which exhibits extremely high specificity for IHC in both mice and non-human primates (NHPs). Based on this vector, a dual-vector OTOF expression system was designated as EA0010.

Results: EA0010 was injected into inner ear through round window in adult wild type mice and NHPs for safety verification. EA0010 rescued hearing of humanized adult OTOFQ829X/Q829X mice. To evaluate the effectiveness of EA0010 in patients with severe-to-profound hearing loss caused by OTOF gene mutations, we designed a single-center, single-arm, open-label, dose-escalation study. From Dec 2024, nine patients have been enrolled in this study.

Conclusions: Preliminary clinical results demonstrate that EA0010 effectively restores auditory function, especially in restoring high-frequency hearing, with no serious adverse event. Detailed results will be presented.

SU36. A Novel AAV Vector-Based Gene Therapy for Two Adult Rodent Models of DFNB1

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Background: GJB2-related autosomal recessive nonsyndromic HL (DFNB1) is the most common genetic cause of congenital sensorineural HL worldwide, accounting for up to half of all cases in some populations. It can also cause later-onset HL, even after 40 years of age. GJB2 encodes connexin-26 (Cx26), a gap-junction channel protein critical for metabolite and ion homeostasis, cochlear development, and sensory hair cell function and survival. Hundreds of pathogenic GJB2 variants have been identified, ranging from large deletions to missense and loss-of-function mutations, with clinical outcomes spanning mild to profound deafness.

Methods: We developed AAV-DS1, an AAV vector optimized for DFNB1, designed for broad coverage of Gjb2-expressing cells in the inner ear of mouse, rat and minipig, while minimizing transduction of hair cells. AAV-DS1 with GJB2-specific promoter to drive the expression of wild-type GJB2 (AAV-DS1-GJB2) was delivered into the inner ears of adult Gjb2-mutant mice and rats via round window (RW) injection. Ongoing studies investigated efficacy, dose-response, correlation between ABR responses and transduction efficiency, early biodistribution, and toxicology in mice. In parallel, AAV-DS1-GJB2 was administered to minipigs using a clinically relevant surgical approach and medical device. Tolerability and biodistribution were assessed, and AAV tropism was characterized by immunoconfocal analysis and in situ hybridization on cochlear whole mounts and cryosections.

Results: A single intracochlear injection of AAV-DS1-GJB2 in adult Gjb2-mutant rats and mice resulted in a significant, dose-dependent improvement in auditory thresholds as early as 2 weeks post-injection. In minipigs, ABR thresholds and DPOAE amplitudes remained within the normal range 6 months post-surgery, confirming local tolerability. Biodistribution was largely restricted to injected ears. In WT mice, AAV-DS1-GJB2 was well tolerated, with no ABR threshold shifts up to 6 months and evidence of long-term transgene expression. Across rodents and minipigs, anti-HA labeling confirmed broad targeting of nearly all GJB2-expressing cell types without adverse functional impact.

Conclusions: AAV-DS1-GJB2 efficiently targets endogenous GJB2-expressing cochlear cells, with demonstrated efficacy and favorable safety in rodents and minipigs. These findings provide strong support for advancing AAV-DS1-GJB2 into clinical translation—enabling studies as a novel gene therapy for GJB2-related hearing loss.

SU37. Triple Antisense Oligonucleotide-Mediated Exon Skipping Targeting a Large Deletion in USH2A

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Background: Usher syndrome is an inherited autosomal recessive condition that accounts for about 50 percent of all hereditary deaf-blindness cases. There are three types of Usher syndrome, of which Type 2 is the most common. USH2 is characterized by moderate to severe sensorineural hearing loss, nyctalopia, and progressive loss of vision. The most common form, USH2A is caused by mutations in the USH2A, a 15.7 kb gene, that encodes for the protein usherin, expressed in cochlear hair cells and photoreceptor cells.

A patient at Boston Children's Hospital was diagnosed with USH2A. Genetic testing revealed two pathogenic mutations: an in-frame deletion of exons 61-63 that results in improper protein folding and a single base-pair deletion leading to the production of a truncated protein. Using Alpha Fold, we structurally analyzed the protein harboring the three-exons deletion and determined that skipping an additional three exons upstream of the deletion may restore proper protein folding and function. As such, we propose to develop antisense oligonucleotides (ASOs) that target these three exons and lead to removal of the region encoded by six consecutive exons, exons 58 to 63. This shortened protein excludes part of fibronectin (FN) domain FN22, all of FN23 through FN30, and part of FN31. The objective of this project is to determine whether the larger USH2A truncation restores function.

Methods: Exon skipping is a method in which ASOs are used to prevent the interaction between mRNA and the ribosome to elude translation. We designed several ASOs targeting splice sites in USH2A exons 58, 59, and 60, and assessed their skipping in retinoblastoma cells at a concentration of 200 nM for 48 hours. Skipping was evaluated by RT-PCR and sequencing. Patient-derived induced pluripotent stem cells (iPSCs) have also been generated using blood sample from the proband. USH2A expression and splicing in the USH2A iPSCs line is being assessed via RT-PCR.

Results: Primers were designed and validated in RB1 cells. RB1 cells were transfected with ASOs at 200 nM for 48 hours and subsequently harvested. RT-PCR was performed with the different primer sets. We validated skipping of exons 58 and 59 and are currently assessing other ASOs targeting exon 60. We are also assessing multiple exon skips combining different ASOs. Additionally, we are assessing mutant iPSC lines by RT-PCR to confirm splicing in absence of ASOs. We are also developing iPSCs derived inner ear and retinal organoids to test validated ASOs in the context of the three exons deletion.

Conclusions: This strategy is novel in that we are assessing skipping of 3 exons in the presence of another 3-exon deletion. We predict that removal of a large portion of the protein encoded by USH2A will lead to expression of a shorter, yet functional protein.

SU38. AAV Delivery of Cib2 Restores Hearing and Vestibular Function in Cib2 Ko Mice

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Background: Calcium and integrin-binding protein 2 (CIB2) binds to TMC1, the pore forming complex of the mechanotransduction channel (MET) and is essential for hearing. Mice lacking both Cib2 and Cib3 double knockout mice also show impaired vestibulo-ocular reflexes, head bobbing, and poor balance. Cib2 and Cib3 single knockout mice appear phenotypically normal; however, some reports of subtle progressive balance phenotypes have been reported in single KO mice by 9 months of age, along with stereocilia disorganization that may be associated with progressive degeneration. Patch clamp recording from vestibular hair cells suggest that Cib2 and Cib3 are functionally redundant in regulating MET. However, RNA expression of Cib2 is predominantly in striolar hair cells whereas Cib3 in extrastriolar hair cells.

Methods: Here we use vestibular evoked myogenic potentials (VsEP), rotarod, open field, and infrared palm reader analysis of Cib2 and Cib3 single KO mice to assess balance phenotypes in mice. We also used auditory brainstem response (ABR) to examine restoration of auditory function in injected mice. Hairpin chain reaction fluorescent in situ hybridization was used to assess Cib2 and Cib3 expression relative to the Ocm⁺ striolar domain and quantify in cellular resolution. RT-qPCR was completed to assess the relative distribution of Cib2 and Cib3 separately in utricles, saccules, crista, and cochlea to compare to HCR quantitative analysis. Functional assessment of mechanotransduction in Cib2 KO and Cib3 KO tissue was completed using FM1-43 labeling of acutely dissected tissues.

Results: Here we show that Cib2 KO and Cib2/3 DKO mice fail to produce VsEP responses to head jerk stimuli, whereas Cib3 KO mice have normal VsEP responses under the same conditions. Unilateral neonatal utricle injection of AAV is sufficient to restore Cib2 KO VsEP

and ABR responses to near WT thresholds in both ears. Similar to other groups, we identify no significant behavioral balance phenotype in Cib2 or Cib3 single KO mice. HCR-FISH shows non-redundant expression patterns of Cib2 in the striola and Cib3 in the extrastriola in the utricle and saccule. Similarly, Cib2 KO tissue shows FM1-43 dye absent in the striola of the vestibular maculae.

Conclusions: Taken together, these data indicate that Cib2 expression in the striola is necessary for VsEP generation. Additionally, despite its expression in vestibular organs, Cib3 it cannot compensate for loss of Cib2 when it comes to VsEP generation or striolar MET function. These results suggest that Cib2 and Cib3 are not functionally redundant in the vestibular system. Despite the regional differences in their expression, our behavioral phenotyping does not indicate a definitively distinct role for Cib2 versus Cib3 in balance. Thus, the underlying reasons for the non-overlapping expression and function of Cib2 and Cib3 remain an interesting area for future study.

SU39. Investigation of Vestibular Dysfunction Requires Renewed Research Enthusiasm

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Background: Hearing, balance, ocular gaze, head position, baroreflexes, and spatial memory all depend on functional input from structures of the inner ear to the brain. Close examination of scientific research output based on anatomical search terms of peripheral inner ear structures shows an obvious lag in areas of vestibular research progress in areas of academic publication, grant funding, and clinical trial development.

Methods: We utilized data mining of publicly available records (PubMed, NIHReporter, and ClinicalTrials.gov) to assess the rate of inner ear research output quantitatively. PubMed and NIHReporter searches included keyword terms separated by Boolean operators to focus on either auditory or vestibular anatomical peripheral structures with suffixes excluded for more inclusive output metrics. Similarly, we queried clinicaltrials.gov with additional search terms included pertaining to symptoms of inner ear dysfunction and names of syndromes. Query results were categorized into either vestibular (e.g. dizziness, vertigo, imbalance, etc.) or auditory (e.g. hearing, deafness, presbycusis, etc.). We combined these computational approaches with systematic review of published literature including patient survey data to understand the prevalence of hearing loss versus dizziness. We also reviewed reported cost estimates from multiple studies regarding unexplained falls to understand the relative cost of untreated vestibular dysfunction.

Results: PubMed data show that the expansive growth period of inner ear research by publication output from 1950 to 2024, particularly in cochlear research. In contrast, fewer publications contain peripheral vestibular anatomical terms ranked by frequency: saccule > utricle > otolith > semicircular canal > vestibular labyrinth > crista ampullaris > vestibular macula. Publications containing auditory terms ranked by frequency: cochlea > organ of corti > spiral ganglia > basilar membrane > stria vascularis > tectorial membrane > Reissner's membrane. NIHReporter queries from 1990-2024 reveal thousands less grants are awarded to projects focused on vestibular research compared to auditory/cochlear research. Unsurprisingly,

the total dollars spent on auditory/cochlear research surpasses vestibular research investment by a significant margin. Interestingly, our review of various patient survey studies show prevalence estimates between dizziness and hearing loss to be comparable.

Conclusions: Vestibular research consistently receives less public attention in the form of published research output, grant funding, and clinical trial development. While there are unique challenges associated with diagnosing patients with vestibular dysfunction, our prevalence estimates of dizziness appear comparable to hearing loss. During literature review, it became clear that vestibular dysfunction had far worse impacts on patients' quality of life and may have higher diagnostic and treatment costs than hearing loss as well. Despite a substantial need for clinical diagnostic and therapeutic interventions for vestibular dysfunction, research in this area is severely limited. A renewed enthusiasm among the next generation of trainees to investigate the vestibular system may help facilitate broader understanding of the inner ear and produce interventions for patients.

SU40. Catalyzing Hearing Research With the Gene Expression Analysis Resource (umgEAR.org)

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Background: The gene Expression Analysis Resource (gEAR, umgear.org) is an online portal for multi-omic data visualization, sharing, and discovery, and continues to be an invaluable public resource for the hearing research community. gEAR allows for visualization, investigation, and reuse of sequencing datasets in a user-friendly web interface. The availability of hearing datasets in gEAR continues to grow with 1,299 datasets, 2,557 unique users, and over 3.75 trillion individual datapoints.

Methods: The proliferation of single cell/nuclei, epigenomics, multiome, and spatial transcriptomics techniques in the hearing research field has led us to incorporate additional visualization and analysis tools which provide users the ability to explore and analyze these datasets using only a web browser with no coding required. Expanded epigenomic tools allow users to quickly visualize open chromatin regions and methylation data alongside gene expression and other dataset displays.

Results: In addition to the range of visualization and search functionality offered via gEAR's new Epigenetic Explorer tools, epigenetic datasets in gEAR now dynamically link to the popular UCSC genome browser for even more analysis options. New spatial tools allow for the upload and interactive exploration of data from all major commercially available spatial platforms (e.g. 10X Visium and Xenium, CosMx and Curio, and more). Similar to other tools in gEAR, the spatial layouts now can be viewed within a dataset collection allowing for correlated views with other popular datasets.

Conclusions: In contrast with other online data visualization tools, a major strength of the gEAR platform is the ability to visualize, curate, and explore multiple datasets and data types side-by-

side. New options for publication landing pages (new pages with custom content to highlight your manuscript and link to all your data), dataset collection customization, and easily implementable transfer learning tools help researchers dramatically increase the visibility, interpretability, and reuse of their sequencing datasets and explore them alongside other cutting-edge hearing datasets. For users visiting gEAR for the first time, gEAR now includes interactive walkthroughs introducing users to the major functionalities of the platform and use of individual tools. gEAR is supported by the National Institute on Deafness and Other Communication Disorders (NIDCD), Hearing Health Foundation (HHF), and the Hearing Restoration Project (HRP) to promote discovery in hearing research.

SU41. Data-Driven Subtyping of Tinnitus Patients Using Deep Embedded Clustering

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Background: Tinnitus is a heterogeneous auditory disorder characterized by diverse clinical manifestations and variable therapeutic responses. While Tinnitus Retraining Therapy (TRT) has been widely implemented, its outcomes differ substantially among patients, reflecting the multifactorial nature of tinnitus involving auditory, psychological, and neurobiological dimensions. Conventional classification systems often fail to capture this complexity, limiting the precision of diagnosis and the prediction of treatment outcomes. Deep learning, particularly deep embedded clustering, provides an opportunity to identify latent structures within multidimensional clinical data and uncover novel subtypes of tinnitus that may guide personalized therapy. The present study aimed to utilize a deep embedded clustering approach to stratify tinnitus patients undergoing TRT into data-driven subtypes, characterize their clinical and audiometric profiles, and examine differential therapy outcomes across subgroups.

Methods: A retrospective dataset of patients treated with TRT was analyzed, including tinnitus handicap inventory (THI) scores, visual analogue scale (VAS) ratings of loudness and annoyance, audiometric thresholds across frequencies, tinnitus psychoacoustic features, psychological stress (BEPSI) and depression (BDI) indices, and therapeutic histories. Representation learning was performed using an autoencoder to reduce high-dimensional inputs into latent features, which were subsequently clustered using k-means in the embedded space. Subgroup characteristics were examined using descriptive statistics and visualized in latent manifolds. Longitudinal treatment responses, assessed through serial THI and VAS scores, were modeled to evaluate the clinical validity of the identified clusters.

Results: Deep embedded clustering revealed three clinically distinct patient subtypes. The first cluster was defined by frequency-specific hearing loss with moderate tinnitus severity, the second by high psychosocial burden with elevated stress and depression indices, and the third by predominantly tonal tinnitus with relatively preserved hearing. These subgroups demonstrated differential TRT trajectories: patients in the psychosocially burdened cluster exhibited slower recovery and higher residual symptoms, whereas those with tonal tinnitus and minimal hearing impairment showed more rapid and sustained improvement. Feature importance analyses further indicated that baseline THI, stress levels, and high-frequency hearing thresholds were the strongest predictors of subgroup membership and therapeutic outcomes.

Conclusions: This study demonstrates that deep embedded clustering can uncover hidden subtypes of tinnitus patients beyond conventional diagnostic categories. By integrating heterogeneous clinical, audiometric, and psychological features, this data-driven approach provides mechanistic insights into tinnitus heterogeneity and offers a framework for personalized management. Importantly, the identification of subgroups with distinct TRT outcomes underscores the clinical utility of artificial intelligence in guiding patient-specific therapy. These findings highlight the potential of AI-driven diagnostic and therapeutic platforms to bridge complex clinical data with translational neuroscience, advancing the precision of tinnitus care and contributing to a deeper understanding of its underlying pathophysiology.

SU42. Minipig Model Development for Hearing Research

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Background: The inner ear drug, biologics, and device development field has been limited by the lack of large-animal models to effectively bridge the gap between rodents and humans. Here we propose the miniature pig (Sinclair NanoPig) as an alternative large animal model in translational hearing research. Compared to commonly used large animal models such as dogs, cats, and some NHPs, the minipig cochlea dimensions are more similar to humans, enabling preclinical studies that use human-relevant dose volumes for studying drug distribution in the cochlea as well as human-sized implantable devices. Further, use of minipigs as a large animal model in biomedical research is cost-effective and in accordance with recent regulatory guidance to limit the use of NHPs in preclinical research. The minipig holds great promise as a better translational model for hearing research, but in order to expand their use in preclinical hearing research studies, there is a need for refining surgical access for delivery and sampling as well as development of normative audiometric datasets and behavioral audiometry methods that mirror clinical outcome measures of sound perception and audibility.

Methods: We used minipigs to develop an operant sound detection task utilizing a custom touchscreen device. Animals were trained to press illuminated boxes on a touchscreen in response to sound or silent stimuli in a two-alternative forced choice task. In addition to behaviorally assessed hearing thresholds, we have generated normative datasets for both bone- and air-conduction ABRs. Preliminary pharmacokinetic studies attempted perilymph sampling from the cochlear apex but found the approach technically difficult. Simulations showed that sequential perilymph sampling from the lateral semi-circular canal, as used extensively in guinea pigs, provides sufficient detail to document drug distribution.

Results: Through this method development study, we have demonstrated the feasibility of a touchscreen sound detection task in the minipigs and have refined local surgical access surgical approaches for drug delivery and sampling. We have also optimized key parameters for DPOAE and ABR measurement in mini pigs.

Conclusions: The minipig is an alternative large animal model that can effectively bridge the translational gap between rodents and humans due to the similarity in cochlear dimensions and anatomy to the humans. Optimizing perilymph sampling procedures and ABR and DPOAE measures in the minipig and developing behavioral assays that closely approximate outcome

measures in humans such as the audiogram or speech-in-noise tests, further aids in the translational validity of the minipig in auditory preclinical research.

SU43. Universally Controversial Stimuli Reveal That Adversarial Robustness Improves DNN Prediction Accuracy Across the Entire Human Auditory Cortex

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Background: Sensory neuroscientists seek to develop models that replicate the computations of sensory systems and predict responses to complex stimuli. A key challenge is that distinct computational models often make similar predictions for complex natural stimuli, such as speech, masking potentially important computational differences. Prior research found that artificial deep neural networks (DNNs) trained on challenging auditory tasks learn features that predict much of the human cortical response to natural sounds. However, the decisions of DNNs can diverge sharply from human behavioral decisions. For example, standard DNNs make confident categorization judgments for noise-like sounds that are completely unrecognizable to a human observer. Training DNNs to be robust to adversarial attacks better aligns model decisions with human judgments. Despite this behavioral difference, standard and robust DNNs make nearly identical predictions for human cortical responses to natural sounds. Here, we develop a new approach for model comparison to investigate these seemingly contradictory results.

Methods: We developed a method to synthesize sounds that generate universally controversial predictions across the entire human auditory cortex and leveraged this method to compare standard and adversarially robust DNNs. Using fMRI data from 30 participants listening to 165 natural sounds (2 seconds each), we fit voxel-wise encoding models by linearly mapping DNN layer activations to fMRI voxel responses, separately for a standard and robust DNNs trained to recognize spoken words. We then developed a synthesis algorithm that generated a single set of 60 sounds that produced uncorrelated predictions across all auditory voxels. Our synthesis procedure successfully generated perceptually diverse, complex sounds—some speech-like, others tonal or noise-like. We then measured fMRI responses to these controversial sounds along with 120 natural sounds from 9 new participants.

Results: We found that standard and robust DNNs predicted fMRI responses to natural sounds with nearly identical accuracy. Moreover, the predictions from standard and robust models were themselves very similar for natural sounds, virtually guaranteeing similar neural prediction accuracy. In contrast, we found that controversial sounds produced uncorrelated model predictions throughout the entire human auditory cortex, even in participants whose data was not used to synthesize the sounds. We found that the robust model maintained high prediction accuracy for measured fMRI responses to the universally controversial sounds, while the standard model's accuracy dropped to near zero. We also found that natural and controversial sounds share a low-dimensional neural manifold along which standard and robust DNNs make highly controversial predictions.

Conclusions: We provide a general-purpose method to synthesize stimuli that distinguish competing sensory models. It is applicable to virtually any model, modality, or recording

technique. Our results demonstrate that adversarial robustness substantially improves neural prediction accuracy and enables more effective population control along the neural manifold.

SU44. A Multi-Channel Integrated Auditory Function Test System

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Background: ABR is the gold standard for objective hearing assessment of animals and people. The extensive use of genetically engineered mice in hearing research has created an urgent demand for the ability of ABR high-throughput testing.

Methods: We developed a four-channel ABR system enabling simultaneous testing of four subjects, integrating distortion product otoacoustic emission (DPOAE), cochlear microphonic (CM) and endocochlear potential (EP) detection functions.

Results: The system adopts high-speed ADC chip, which can obtain DPOAE and CM data above 32kHz. For the detection of various peak parameters (amplitude and latency) of mouse ABR, the performance is equivalent to that of the industry standard equipment TDT, and the test efficiency is improved by three times

Conclusions: This multifunctional test platform meets the current high-throughput ABR testing requirements, and provides excellent efficiency and expanded functional evaluation for the scalable high fidelity auditory phenotype in the next generation research.

SU45. Irregular Afferent Pathways Associated With the Striola Are the Peripheral Origin of Motion Sickness

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Background: At present, the pathological mechanism of motion sickness (MS) focuses on the mismatch between the real sensory input and the self-motion estimation predicted by the "internal mode". Drugs for the treatment of MS have obvious side effects due to a wide range of central action targets. Basic medical evidence shows that the pathological origin of MS can be traced to the abnormal signal input of vestibular receptors. Can we establish an interpretation model of the mechanism of MS through the analysis of the regulation path of peripheral vestibular input?

Methods: In this project, we studied the vestibulo-ocular reflex (VOR) of CIB2^{-/-} and CIB3^{-/-} transgenic mice expressed in the striola and extra-striola zone of otoliths, including VOR-SPN (Slow phase nystagmus) and VOR-FPN (Fast phase nystagmus). After that, MS in mice was induced by biaxial rotation, and the severity of MS was evaluated using real-time body temperature monitoring (core body temperature and tail temperature), VOR decline after rotation, and swimming posture control after rotation.

Results: (1) VOR-SPN mainly reflect the function of extra-striatal zone, which shows that the slow phase eye movements of aVOR (angular VOR) and OVAR (Off-vertical axis rotation) in

CIB3^{-/-} mice are significantly reduced. CIB2^{-/-} mice have no obvious vestibular dysfunction symptoms, including VOR and VSR.

(2) Compared with the control group C57 BL/6J mice, CIB2^{-/-} mice don't show significant thermoregulatory response during rotational stimulation, and their VOR efficacy was higher than that of the control group after rotation, with normal swimming posture.

Conclusions: The irregular afferent pathway associated with hair cells in the striola zone is the main vestibular peripheral source of MS. This discovery precisely locates the origin of MS from the macroscopic vestibular system to the sub organ structure - the striola zone. Thus, we will shift the targeted diagnosis and treatment of MS from the central system to regulating specific peripheral signaling pathways.

SU46. Resurgent Sodium Currents and Nav1.6 Expression in Vestibular Calyx Afferent Terminals

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Background: Vestibular afferent neurons relay signals from hair cells to the brain. Calyx-only afferents innervate type I cells, bouton afferents target type II cells, and dimorphic afferents contact both hair cell types. Firing characteristics vary by zone, with dimorphic afferents in the peripheral zone (PZ) showing highly regular repetitive activity. Ionic conductances, including sodium currents—transient (INaT), persistent (INaP), and resurgent (INaR)—may shape firing properties. INaR, generated by an open-channel block mechanism supports repetitive firing in select neurons, but its role in vestibular afferent signaling is unclear. We therefore used whole-cell patch-clamp to investigate INaR in vestibular calyces and modeling and immunohistochemical approaches to investigate underlying channels.

Methods: Cristae were extracted from Mongolian gerbils (P30-42), embedded in agarose, and sliced at 100 μ m. The central zone (CZ) and PZ regions were identified, and calyx terminals distinguished by their cup-shaped morphology. Whole-cell patch-clamp was used with K⁺- or Cs⁺-based intracellular solutions. A series of voltage protocols was applied to characterize INaT and INaR in voltage clamp. Action potentials were recorded in current clamp using K⁺-based electrode solutions. Na⁺ currents were modeled with a Markov kinetic scheme (Hong et al. 2018). For immunohistochemistry, slices were fixed and stained with anti-CASPR1 and anti-Nav1.6 antibodies and fluorescent images viewed on a confocal microscope.

Results: In whole-cell recordings all calyces expressed INaT. INaR was present in a subset of calyces, with 19 out of 38 PZ calyces (50%) and 4 out of 16 CZ calyces (25%) exhibiting INaR. Both current components were blocked by tetrodotoxin (500 nM). Preliminary data demonstrate that INaT in calyces expressing INaR activate at more negative potentials compared to those lacking INaR suggesting different underlying subunits. INaR amplitude depended on the duration and voltage of the preceding depolarizing step: brief (10 ms) or larger (+30 mV) depolarizing prepulses enhanced INaR compared to longer (50 ms) or smaller (−10 mV) steps, consistent with open-channel block and slow inactivation dynamics. INaR⁺ calyces exhibited more depolarized half-inactivation potentials for the INaT ($V_{1/2} = -72.4$ mV) than INaR[−] calyces

($V_{1/2} = -85.7$ mV). INaR facilitated faster recovery from both open-channel block and inactivation, and our computational model replicated voltage clamp findings. In current clamp, calyces with INaR displayed multiple spikes whereas those without INaR typically showed single evoked spikes. Anti-CASPR1 stained calyces and heminodes adjacent to anti-Nav1.6 staining below the neuroepithelium.

Conclusions: We found that INaR is prevalent in PZ calyces of the crista. Calyces with INaR exhibit faster recovery from inactivation and open-channel block, more depolarized half-inactivation of INaT, and repetitive firing. Nav1.6 channels may contribute to the generation of INaR within dimorphic calyx afferents. Understanding the heterogeneity of Na⁺ channels and firing mechanisms in vestibular afferents could guide treatment for vestibular dysfunction.

SU47. Effect of Modulus Presentation on the Magnitude Estimates of Angular Velocity

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Background: Magnitude estimation is a psychophysical method in which participants assign numerical values to physical stimuli. This method can be used to measure the perceived intensity of vestibular sensory experiences, such as angular motion. A reference stimulus, or modulus, is commonly used as an initial reference to which a specific number is assigned. Subsequent stimuli are then assigned numbers that reflect the participant's perceived magnitude relative to the modulus. While use of a modulus during magnitude estimation of various sensory experiences was used in the original method by S.S. Stevens in the 1950's, not all applications of magnitude estimation use a modulus in the same way. To date, the effect of modulus presentation on magnitude estimation of vestibular stimulation is not understood. The purpose of this study was to examine whether the scaling of a sinusoidal harmonic acceleration stimulus presented at varying peak velocities changed with the presentation of the modulus.

Methods: Twenty-one healthy adults (mean age = 20.5 years) estimated the magnitude of a single cycle sinusoidal harmonic acceleration stimulus presented at .16 Hz in 9 randomly presented peak velocity conditions (10-90 dps). A modulus was presented to all participants and assigned a value of 50. Participants were randomly assigned to one of three modulus groups: (1) modulus presented once at the beginning, (2) modulus presented at the beginning and again at midpoint, or (3) modulus presented before each condition. The dependent variable was the slope of the psychophysical function.

Results: Across groups, perceived angular velocity followed a log-linear function consistent with Stevens' Power Law. The exponents of perceived angular velocity varied between .72 and .81. Statistical analyses revealed no significant group differences.

Conclusions: The psychophysical function obtained by the method of magnitude estimation was not significantly influenced by the modulus presentation as a compressive scaling function was observed regardless of modulus presentation frequency. The results support the use of a single modulus, presented at the beginning. Future work should examine conditions without a modulus (i.e. "free modulus" where participants choose their own reference numbers) and whether there are interactions between the magnitude of the modulus and the psychophysical function. Future work should also examine if these results hold true in different participant cohorts, particularly

older participants or those with vestibular loss. Key take-away: The frequency in which a modulus was presented did not significantly affect the psychophysical function obtained by the method of magnitude estimation of angular motion.

SU48. Fall Risk in Underserved Populations

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Background: Falls are a leading cause of morbidity among older adults, yet disparities in fall risk remain poorly understood in underserved populations. Standardized tools such as the CDC STEADI (Stopping Elderly Accidents, Deaths, and Injuries) provide a validated framework for community-based screening but do not incorporate audiologic or vestibular risk factors. In the Harrisonburg, VA community, approximately 20% of residents identify as Hispanic, a proportion nearly double the statewide average, making the inclusion of this population especially important for fall risk screening. The proposed study will enhance the STEADI model by integrating vestibular and hearing assessments along with social determinants of health, including health literacy, using materials available in both English and Spanish. Specific aims are to: (1) assess the prevalence of fall risk among Hispanic and non-Hispanic White adults ≥ 40 years old, (2) determine the prevalence of previously undiagnosed benign paroxysmal positional vertigo (BPPV) in underserved communities, and (3) construct a predictive model of fall risk incorporating auditory, vestibular, and social determinants of health with a focus on falls risk among Spanish-speaking Hispanic adults over age 40.

Methods: Participants from the Harrisonburg, VA community completed the CDC STEADI screener, pure-tone audiometry, tympanometry, vestibular assessments (Dix-Hallpike, vHIT, VEMPs, Rotary Chair), and health literacy/social determinants questionnaires. Recruitment occurred through local community centers, churches, and the university.

Results: Data collection is ongoing and expected to wrap up fall 2025 with a sample size of 80. Preliminary pilot data (N = 14; mean age = 54.6 years) suggest that fall risk prevalence is higher among Hispanic participants compared to non-Hispanic White adults. The CDC STEADI identified 43% of Spanish-speaking Hispanic participants as at risk, > the national average. BPPV prevalence in the Hispanic participants was higher than national estimates (14% vs. 3%). Logistic regression analyses indicated that participants with BPPV were approximately five times more likely to be classified as at risk for falls compared to those without BPPV, and participants with low health literacy had 1.5 times greater odds of being at risk relative to those with adequate health literacy. These findings suggest that both vestibular dysfunction and health literacy are important contributors to fall risk and may be underrecognized in underserved populations.

Conclusions: Early findings suggest that using audiologic measures such as BPPV screenings and social determinants of health such as measuring health literacy, can add predictive value to determining who is at risk of falls. Incorporating audiologic and vestibular assessments improved detection and revealed disparities in risk factors. These preliminary results support larger-scale studies to validate predictive models integrating sensory and social determinants of health.

SU49. The VGluT1-Cre Mouse Targets a Subpopulation of Central Zone Afferents in the Mouse Vestibular Epithelia

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Background: In the vestibular nerve, the temporal precision needed to code rapid head movements is determined by neurons firing at irregular intervals, whereas the ability to detect slow head movements sensitively is determined by neurons firing at regular intervals. The dendritic terminals of irregular afferents are predominantly located within the central zones of the sensory epithelia while the terminals of regular afferents are within the peripheral zones (Fernández et al. 1988; Lysakowski et al. 1995). Because afferent firing patterns are tightly linked to central versus peripheral zones, we tested whether ion channel composition contributes to this diversity by using a Vglut1-Cre driver mouse crossed with an Ai14 TdTomato reporter to selectively label and record from ganglion neurons originating in the central zones of vestibular epithelia. Here we report the peripheral innervation pattern of the fluorescent neurons, their co-expression with Calretinin, and their biophysical properties. This work extends our previous report of central-zone innervation by providing an updated account of their molecular and biophysical features.

Methods: The prevalence of Vglut1-Cre positive (TdTomato positive) neurons was quantified in immunolabeled samples of whole-mount epithelia and cryo-sectioned vestibular ganglia from adult (post-natal day (P)60-P70) mice. We used antibodies against Beta-III tubulin (most somata) and Calretinin (sub-population of central zone somata) to evaluate the overlap between fluorescent somata and other sub-populations. Cross-sectional area of each cell was inferred by the boundaries of the Beta-III tubulin signal. Finally, we recorded using perforated patch-clamping methods from disassociated and cultured ganglion somata at two age ranges (P16±1 days) and adult (P60-P140).

Results: In epithelial whole-mounts, TdTomato positive neurons contacted the central zone of vestibular epithelia with both bouton and calyx terminals. In ganglion sections, TdTomato positive somata were found amongst the medium and large somata and constituted approximately 6% of the total population. Approximately two-thirds of the TdTomato positive somata also co-labeled with the central zone marker Calretinin. Patch-clamp recordings demonstrated that the fluorescent neurons have both transient and intermediate firing patterns. They also responded to simulated synaptic noise with irregular responses, qualitatively support the hypothesis that ion channel properties of central-zone fibers account for irregular firing.

Conclusions: The Vglut1-Cre^{*}Ai14 mouse line effectively targets vestibular afferents, with a bias toward irregularly firing central-zone fibers. The biophysical properties of these neurons support their role in generating irregular firing. Ongoing work is characterizing regional variations in ion channels (KCNQ, HCN, and Kv1) that may underlie differences in quantal versus nonquantal transmission. This line offers a promising tool for testing the functional contributions of central-zone neurons through genetic manipulation.

SU50. PROVIDE: Patient-Centered Care for Vestibular Disorders

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Background: Vestibular disorders extend far beyond dizziness and imbalance. They encompass a spectrum of symptoms, ranging from nausea and a loss of equilibrium to cognitive impairments, depression, and reduced quality of life. Current treatments remain limited in both effectiveness and personalization. The PROVIDE Doctoral Network (<https://provide-dn.eu/>), funded under the Marie Skłodowska-Curie Actions (MSCA) within the Horizon Europe framework, addresses this pressing clinical challenge. The goal of the network is to advance patient-centered care for vestibular loss by integrating various methodologies and expertise across disciplines and bridging scientific research with innovative solutions.

Methods: PROVIDE's research activities are integrated across three complementary work packages (WPs) identifying the underlying etiology (WP2), improving characterization (WP3), and bringing innovative treatments of vestibular loss to home use (WP4). Through an interdisciplinary approach, PROVIDE unites expertise from pre-clinical animal models and organoids to novel diagnostic tools to clinical interventions using haptic biofeedback, neuromodulation, and vestibular implants. In total, 22 partners from 12 countries are involved in the training and scientific development of 10 doctoral candidates.

Results: PROVIDE is generating scientific insights that advance the pathway from identification of the individual vestibular disorder etiology to providing personalized treatment. It is also developing new tools and methods that are freely available to all researchers. In addition, PROVIDE is creating openly accessible educational resources and training materials through an online learning platform as well as opportunities to participate in training events.

Conclusions: By promoting equitable and inclusive practices in training, research, and healthcare, PROVIDE is advancing interdisciplinary vestibular research and contributing to the development of sustainable tools to transform the management of vestibular loss and foster knowledge exchange and outreach beyond the network.

SU51. Effects of Prosthesis Stimulation on Compensatory Saccades During the En-Bloc Head Impulse Test in Vestibular-Impaired Monkeys

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Background: The vestibulo-ocular reflex plays a critical role in gaze stabilization. Patients with vestibular loss cannot maintain gaze on a target during head movements because the eyes move with the head. The head-impulse test (HIT) detects this instability by having patients fixate on a target while the head is rapidly and unpredictably rotated to stimulate the semicircular canals. Patients make two types of compensatory saccades during HIT: covert saccades, which occur during head rotation, and overt saccades, which occur after head movement stops. Patients who acquire covert saccades show improved Dizziness Handicap Inventory scores, higher visual acuity, and reduced oscillopsia, likely because retinal slip is minimized and vision is suppressed during the saccade. Learning to generate covert saccades is therefore important for quality of life. To study the neural basis of these movements, we developed a vestibular-impaired monkey model and confirmed use of a saccadic strategy during en-bloc HIT, in which head and body rotate together.

A vestibular neuroprosthesis has been used to replace missing sensory motion information. Although significant progress has been made in surgical implantation techniques and waveform optimization over the past two decades, functional restoration remains incomplete. Novel approaches are therefore needed to achieve full recovery. As a supplement to current strategies, one approach is to understand how the brain integrates and adapts to artificial stimulation delivered alongside natural neural activity, including residual vestibular function and saccades. In this study, we test whether vestibular prosthesis stimulation facilitates saccades during en-bloc HIT and assess its long-term effects.

Methods: One bilaterally vestibular-impaired monkey was tested during en-bloc HIT at a peak velocity of 100°/s. The right horizontal semicircular canal was stimulated with a vestibular prosthesis delivering biphasic pulses at 250 pps.

Results: Vestibular neuroprosthesis stimulation facilitated leftward saccades. Repeated sessions enhanced performance in both stimulation and no-stimulation conditions, and this effect persisted for several days.

Conclusions: Right-ear stimulation drives activity in the right vestibular nucleus, which in turn enhances activity in the left abducens nucleus through excitatory projections and reduces right abducens nucleus activity via inhibitions. During leftward saccades, the right abducens serves as antagonist whereas the left abducens as agonist. As stimulation depressed antagonist activity and enhanced agonist activity, the balance shifted toward easier saccade initiation. Furthermore, the long-lasting effects suggest that stimulation also induces adaptation or plastic changes in neural circuits that control gaze stabilization.

These findings indicate that vestibular prosthesis stimulation may facilitate recovery by enhancing covert saccade initiation.

SU52. Defining Contributions of the Vestibular Nuclear Complex (VNC) to Vestibular Short Latency Evoked Potentials (VsEPs) of the Rat

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Background: The vestibular system is crucial for posture, gait, and the perception of head and body position in space. Our lab has shown that linear jerk stimulation of irregular vestibular afferents activates central vestibular pathways and is measurable with vestibular-short latency evoked potentials (VsEPs; Holt et. al, 2025). Previous lesion studies in chickens have shown that P2 and later peaks of the VsEP waveform reflect activity of central pathways (Nazareth and Jones, 1997). However, the contribution of the vestibular nuclear complex (VNC) to mammalian VsEP waveform peaks is not well understood. Designer receptors exclusively activated by designer drugs (DREADDs) are genetically engineered receptors used to modulate neuronal activity. These receptors are only activated upon administration of an exogenous ligand. We expressed inhibitory DREADDs in the VNC to initially determine whether the VNC contributes to later VsEP peak(s).

Methods: Sprague-Dawley rats received bilateral VNC injections of an inhibitory DREADD viral vector (AAV2-CAG-hM4Di-P2A-mCherry-WPRE) via stereotaxic surgery. Craniotomies were sealed with dental cement, and a ceramic nut was fixed at Bregma to allow attachment to

the shaker arm for jerk stimulation. Three - 4 weeks after the surgery, VsEPs were recorded across two sessions, at least one week apart. Each session included two blocks of 2,000 jerks (1,000 up, 1,000 down, ~3,300 g/s). During session one, animals received saline (IP) before block one and deschloroclozapine (DCZ; DREADD agonist) before block two. During session two, saline was given prior to both blocks to control for the potential effect of repeated stimulation. VsEP amplitudes and latencies (P1, P2, P3) in each session were analyzed and compared across blocks. To determine which brain regions expressed the fluorescent DREADD receptors, animals were transcardially perfused. Brains were postfixed, cryoprotected, serially sectioned (40 μ m), and imaged.

Results: During the first session, P3 amplitude was significantly reduced in the block following DCZ administration compared to the saline block ($p < 0.05$, paired T-test). There were no significant differences in P1, P2, and P3 amplitudes between blocks in session 2. Administration of DCZ also increased P2 response latencies compared to the saline block in session one ($p < 0.05$). No significant differences were found between the saline blocks in session two. To confirm DREADD expression, we imaged histological sections through the VNC.

Conclusions: Our data show that partially inhibiting rostral VNC activity reduces P3 neuronal synchrony and delays P2 and P3 response times. The changes observed in synchrony and process speed reflect VNCs contribution to later VsEP waves. These results advance the interpretation of VsEPs as a tool for assessing both peripheral and central vestibular function. Future studies should assess the effects of rostro-caudal position and VNC subdivisions to the VsEP waveform.

SU53. Characterization of Auditory and Vestibular Consequences in a Preclinical Single and Repetitive Blast Model

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Background: Blast explosions can inflict significant damage on the auditory and vestibular systems. Acute symptoms following even mild blast exposures can include tinnitus, hearing loss, dizziness, and disorientation, whereas high rates of chronic sensorineural hearing loss and vestibular dysfunction are reported in the military population. In this study, we directly compare outcomes following single vs repetitive exposures to investigate progressive inner ear functional loss caused by low-intensity blasts.

Methods: Brown Norway rats (n=8; 14-18 weeks) were randomized to single-blast (8–12 psi), repetitive blast (10 total blasts, once daily) or sham (no blast). Anesthesia for the blast was achieved with isoflurane supplemented with O₂. Auditory brainstem response (ABR; 4, 8, 16, 24 kHz) and cervical vestibular evoked myogenic potential (cVEMP; 1,8 kHz) amplitude, latency and thresholds are collected at baseline, blast day (BD) 5 and BD10, post-blast day (PBD) 7, PBD14, and PBD 28. Tympanic-membrane integrity was examined prior to electrophysiological recordings. Gait kinematics, including fine movement and posture were analyzed across the groups.

Results: Both single-blast and repetitive-blast groups exhibited a significant shift in ABR and cVEMP thresholds compared with sham controls. The elevation in thresholds was accompanied by changes in amplitudes and latencies consistent with acute auditory and vestibular dysfunction. By PBD28, animals in the single-blast group demonstrated recovery, with ABR and cVEMP thresholds returning toward baseline values. In contrast, the repetitive-blast group exhibited persistent ABR and cVEMP threshold elevations at PBD28 compared to both sham and single-blast animals.

Conclusions: These findings indicate that, while both single and repetitive mild blast exposures disrupt peripheral auditory and vestibular function, recovery diverges across these groups over time. Single-blast animals show transient deficits that are partially resolved within 28 days, whereas repetitive-blast animals exhibit sustained auditory and vestibular dysfunction. This suggests that cumulative blast exposure potentially impairs endogenous functional recovery and produces long-lasting sequelae, highlighting the importance of further investigation of its mechanism.

SU54. Identifying Neurons in the Vestibular Nuclei Responsive to Vestibular Evoked Potentials

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Background: The peripheral vestibular system consists of two otolith organs that detect linear accelerations and three cristae which detect angular accelerations. These sensory organs are innervated by the vestibular ganglion that relays peripheral inputs to the vestibular nuclei (VN) located within the brainstem, as well as to the cerebellum. However, the circuitry and identity of the VN neurons are still largely unknown; specifically, how peripheral vestibular information from the end-organs is relayed and processed centrally is poorly understood. An important functional assessment of the striola of the otolith organs is the initiation of compound action potentials known as vestibular-evoked potentials (VsEP), in response to jerk stimuli to the head. Understanding how the VN respond and process inputs from the striola will provide insight into central processing of vestibular information in general. To identify VN neurons responding to VsEP, we examined the expression of c-Fos, an immediate early gene induced during neuronal activation, following jerk stimulation in mice. To further validate that the observed c-Fos expression is associated with VsEP, we performed the same jerk stimuli on Tmie knockout mutants, in which all hair cells lack mechanotransduction and consequently, VsEP is absent.

Methods: We applied a modified VsEP protocol to stimulate the utricle and saccule in anesthetized adult mice using maximum, repetitive linear jerk stimuli continuously for 20 minutes. Control animals also received anesthesia but were not stimulated. Thirty minutes after the end of stimulations, transcardiac perfusion was performed. Then, the brain was dissected, fixed, and cryopreserved. During processing, coronal cryosections of the brainstem at the level of VN were collected. Multiplex RNAscope was performed using probes for c-Fos, paired with Calbindin-II and Neurofilament for VN identification. In WT animals, comparisons were made between stimulated and unstimulated controls. In Tmie^{-/-} experiments, comparisons were made

between stimulated Tmie^{-/-} mutants against unstimulated Tmie^{-/-} and stimulated Tmie^{+/-} littermates.

Results: Stimulated WT mice showed an increase in c-Fos labeled neurons in caudal medial vestibular nuclei (MVN) and descending vestibular nuclei (DVN), compared to unstimulated controls. This increase in c-Fos expression is consistent with the role of striola in mediating postural control. Congruently, such increase of c-Fos⁺ neurons in DVN of Tmie^{+/-} stimulated controls was strongly reduced in stimulated Tmie^{-/-} mice. By contrast, an increase in c-Fos⁺ neurons in the rostral MVN was observed in both stimulated and unstimulated Tmie^{-/-} animals, compared to Tmie^{+/-} littermates.

Conclusions: Results of the Tmie^{-/-} mutants support the conclusion that the increased c-Fos expression found in caudal MVN and DVN after jerk stimuli is associated with VsEP. Currently, we are investigating whether these c-Fos⁺ neurons are excitatory or inhibitory in nature using known molecular markers. We are also characterizing the upregulated c-Fos expression in rostral MVN of Tmie^{-/-} mutants, which is independent of jerk stimuli.

SU55. Dexamethasone-Eluting Cochlear Implantation Reduces Intracochlear CX3CR1⁺ Cellular Infiltration and Foreign Body Response in Long-Term Follow-Up in Mice

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Background: Cochlear inflammation and foreign body response following cochlear implant (CI) surgery may impact clinical outcomes by contributing to delayed loss of residual acoustic hearing and increasing CI electrode impedance. We have previously reported that a dexamethasone-eluting CI (DexCI) reduces macrophages infiltration and the foreign body response when assessed up to 112 days post-CI in mice and reduces CI impedance values in humans in a similar time-frame. It is not known whether these anti-inflammatory and anti-fibrotic

effects persist at clinically relevant longer-term follow-ups. This study aims to assess the effect of the DexCI in reducing the post-CI foreign body response at longer time-points beyond when dexamethasone elution is anticipated to be diminished.

Methods: 10-12-week-old CX3CR1⁺/GFP Thy1⁺/YFP mice (n=6 per group at each timepoint) on

C57Bl6 background with normal hearing, were implanted with a standard or dexamethasone-eluting mouse CI (standard CI, DexCI, respectively) in the left ear via the round window; with the

right ear being an unoperated control. Cochleae were harvested at 224- or 336-days post-CI

and prepared for immunohistochemistry to assess macrophages (CX3CR1), spiral ganglion neurons (Thy1), fibrotic tissue (α -SMA) and nuclei (DAPI). Subsequent Movat's Pentachrome staining was performed to further characterize cochlear fibrosis and neo-ossification and assess for cochlear trauma. The density of CX3CR1+ macrophages was measured in the scala tympani (ST), Rosenthal's canal (RC) and lateral wall (LW) as well as the spiral ganglion neuron (SGN) density and volume of α -SMA-positive fibrotic tissue in the ST for each cochlear turn.

Results: All cochleae implanted with a Standard CI showed robust cochlear fibrosis and neo-ossification at 224- and 336-days. However, DexCI cochlea showed minimal to no foreign body response, except in 2/6 cases at 224-days where basilar membrane translocation of the CI was identified. CX3CR1+ macrophage density and α -SMA-positive fibrotic tissue was significantly reduced in the ST, LW and RC of the basal turn in the mice implanted with DexCIs when compared with standard CIs at both 224- and 336- days post-CI. The qualitative degree of neo-ossification and fibrosis was most pronounced in cases of translocation in the Standard CI group, with the FBR spanning all three scala in instances of translocation versus being confined to the ST in cases without CI translocation.

Conclusions: DexCI reduced macrophage infiltration and fibrosis relative to Standard CI over the long-term (224- and 336- days post-CI), except in cases where basilar membrane translocation was identified. Further, we observe that insertion trauma in the form of CI translocation may amplify the CI foreign body response. These findings show that in a mouse model, DexCI reduces fibrosis well beyond the expected dexamethasone elution window, suggesting the foreign body response mitigation to be a durable effect long-term.

SU56. Systemic Treatment With Antisense Oligonucleotides Restores Vestibular Function in Usher 1C Mice

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Background: Usher syndrome (USH) is a rare genetic disorder characterized by the multi-sensory loss of hearing, balance, and vision. Approximately 2.5% of USH is caused by mutations in the USH1C gene, encoding the protein harmonin expressed in hair cells. While assistive devices such as cochlear implants help manage hearing loss, no therapies exist to restore vestibular function. Here, we studied the therapeutic effects of antisense oligonucleotides (ASO-29) to treat balance dysfunction in an Ush1c c.216G > A knock-in mouse model of USH1C. The ASO-29 targets the 216A mutation to redirect aberrant Ush1c splicing and promote full-length harmonin expression.

Methods: A single dose of ASO 29 was delivered to USH1C mice by intraperitoneal injection at postnatal day 2 (P2) and aged to 6 months. Vestibular function was assessed in ASO-treated and

untreated USH1C mice and wild-type (WT) littermate controls by measuring the vestibulo-ocular reflex (VOR) responses. Eye movements were recorded using a high-speed eye tracking system. Rotational VOR (rVOR) and translational VOR (tVOR) were measured to assess semicircular canal and otolith function, respectively. Gains, phases, and distortion indexes were calculated by performing a fast Fourier transform (FFT) analysis. General motor behavior was assessed by open-field, rotarod, and balance beam analyses. Hearing function was assessed by auditory-evoked brainstem response (ABR) analyses.

Results: At six months of age, untreated USH1C mutant mice had significantly reduced rVORs compared with WT littermates, indicating severe canal function deficits. TVOR gains remain comparable to WT mice. Additionally, untreated USH1C mice showed circling behavior, and significantly increased latency-to-fall on the rotarod and time-to-traverse the balance beam, and significantly elevated hearing thresholds at all frequencies tested compared with WT littermates. ASO-29-treated USH1C mice showed robust rVOR responses corresponding to 56 to 65% of WT levels across the tested frequency range. ASO-treated USH1C mice also showed improved latency-to-fall on the rotarod and time-to-traverse on the balance beam compared with untreated USH1C littermates. ABR analyses showed improved of hearing thresholds in ASO-treated USH1C mice compared with untreated USH1C mice.

Conclusions: Systemic ASO-29 treatment in USH1C mutant mice led to a significant restoration of vestibular canal function, vestibular behavior, and hearing function. These results demonstrate the therapeutic potential of ASOs to treat balance deficits associated with Usher syndrome. (supported by NIH R01 DC020243-01A1, 1P20GM156708-01) (JH and JL: co-presenter, JL, WZ, HZ co-correspondents)

SU57. Postnatal Role of Jagged1 in the Hair Cells and Supporting Cells of the Vestibular System

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Background: The vestibular system, composed of the utricle, saccule, and semicircular canals, is essential for balance and spatial orientation. Sensory function within this system relies on two types of hair cells (HCs) (type I and type II), which detect head motions and gravity. Supporting cells (SCs) encase HCs, providing structural support and protecting HCs from damage, much like glial cells support and protect neurons in the central nervous system. Jagged1 (Jag1), a Notch ligand, is crucial for inner ear development, guiding progenitor cell formation, while other Notch ligands mediate the differentiation of progenitor cells into HCs and SCs. While Jag1's role in early cochlear and utricular development is well-established, its continued postnatal expression suggests an additional function during maturation. Prior research demonstrated that deleting Jag1 from SCs of the cochlea at birth led to the loss of a SC subpopulation, called Hensen cells, caused a mild hearing loss, and stereocilia deficits in inner HCs (Chrysostomou et al., 2020; Gilels et al., 2022). Previous studies have shown that Jag1 is expressed in the utricle after birth and into adulthood, suggesting its potential involvement in other processes such as postnatal HC addition, maturation, turnover or survival. Here we deleted Jag1 from utricular HCs or SCs at birth to understand its function.

Methods: We generated mouse models to delete Jag1 specifically from HCs or SCs at postnatal day (P) 1 using: Atoh1-CreER:Jag1^{f/f}:Rosa26Tom^{+/f} mice for HC-specific Jag1 deletion, and Plp-CreER:Jag1^{f/f}:Rosa26Tom^{+/f} mice for SC-specific Jag1 deletion. Utricles were immunostained with myosin VIIA to label HCs, SOX2 to label SCs and type II HCs, and Phalloidin to visualize phagosomes and stereocilia bundles. We quantified the number of each cell type at 2 and 4 weeks after the Jag1 deletion using confocal microscopy.

Results: Our preliminary results suggest a decrease in the number of SCs following Jag1 deletion from SCs, suggesting that Jag1 plays a role in maintaining SC survival postnatally. Additionally, when Jag1 was deleted from utricular HCs, we observed an increase in the number of phagosomes that target HCs at both 2 and 4 weeks post-deletion compared to controls. However, our preliminary results showed that there is no significant difference in the number of type I or type II HCs in HC-specific deletion of Jag1. This lack of change may be due to endogenous cellular turnover mechanisms that compensate for the loss of Jag1, preserving the overall HC population. However, a larger sample size and analysis at longer post-deletion periods are needed.

Conclusions: Our findings suggest that Jag1 plays a role in maintaining the survival of SCs in the postnatal utricle. The increase in phagosomes when Jag1 was deleted from HCs also suggests a role for Jag1 in the natural HC turnover process.

SU58. Effects of Tmie Deletion on Vestibular Hair Cells in Mice

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Background: Tmie gene encodes a protein that is crucial for the mechanoelectrical transduction (MET) channel complex in the hair cells in the inner ear (Zhao et al., 2014). In a prior investigation, we characterized the vestibular phenotypes of spinner, a mouse model with a Tmie deletion for human hearing loss DFNB6 (Iriarte Oporto et al., 2025). Vestibulo-ocular reflex (VOR) tests showed that Tmie KO mice exhibited no eye movement responses to head rotation (rotational VOR, rVOR) and substantially reduced responses to head translation (translational VOR, tVOR). Additionally, single vestibular afferent recording showed that substantial reduction in the spontaneous firing rates of both canal and otolith afferents, a decrease in the population of regular canal afferents, and diminished sensitivity of otolith afferents to translation. In this study, we further examined whether loss of vestibular hair cells may underlie the functional deficits we observed.

Methods: Temporal bones were collected from 4 to 7-month-old male and female C57 BL/6 wild-type (WT), Tmie^{+/-}, and Tmie^{-/-} mice. Animals were perfused transcardially with 4% paraformaldehyde, and temporal bones were post-fixed, decalcified in 0.25 M EDTA, and microdissected to isolate cristae and maculae for whole mount processing. Immunolabeling was performed using primary antibodies against Myo7a, Sox2, and OCM. Myo7a specifically labels all hair cells, Sox2 identifies both supporting cells and type II hair cells, and OCM highlights the striolar region of the utricle and saccule and the central region of the cristae. Imaging was

conducted utilizing a Zeiss LSM 880 confocal microscope and image analysis was carried out with Zen Blue software and Fiji/ImageJ.

Results: Preliminary analysis showed no significant differences in overall hair cell density in the saccules and horizontal canals between control and Tmie KO mice. Similarly, OCM-positive cells in the utricle, saccule, and horizontal cristae did not differ between control and Tmie KO mice. Sox2 immunostaining showed increased proportion of Type II hair cells in the central region of the horizontal cristae, and the extrastriolar region of the saccule in Tmie KO mice.

Conclusions: Although preliminary, our morphological analysis indicates that vestibular hair cells are preserved in Tmie KO mice, suggesting that the observed vestibular functional deficits are not attributable to hair cell loss. This contrasts with the auditory system, where Tmie KO mice exhibit clear hair cell degeneration. Ongoing studies are focused on examining the structural integrity of vestibular hair cell hair bundles in Tmie KO mice to further clarify the basis of the functional impairments.

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SU59. (Micro)structure of the Brainstem-Cortical Pathways and Their Relation to Auditory Categorization and Speech-In-Noise Performance

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Background: Successful speech perception requires listeners to bin continuous acoustic information into discrete phonetic categories. Categorization skills vary among listeners; some maintain within-category acoustic information (gradient) while others discard category-irrelevant information (discrete) during perception. Listeners also vary in how consistently they label speech sounds and more gradient/consistent labeling has been linked with better speech-in-noise (SIN) perception. How gross brain anatomy and microstructure (i.e., white matter tract density) of the brain's major speech-language and auditory pathways relates to individual differences in speech categorization and SIN processing has not been explored.

Methods: We assessed individual differences in categorization and SIN comprehension via phoneme labeling and QuickSIN tasks. Diffusion-weighted imaging (DWI) with probabilistic tractography estimated axonal density (quantitative anisotropy – QA) of bilateral arcuate fasciculi and the acoustic radiations. Anatomical segmentation provided estimates of surface area, gray matter volume, and thickness of important frontotemporal auditory-linguistic brain areas (Broca's area) and the midbrain.

Results: Behaviorally, we found QuickSIN scores were correlated with changes in the slope of listeners' identification curves suggesting a more stable categorization listening strategy yielded better (lower) SIN thresholds. DWI data showed that listeners with greater white matter density in bilateral arcuate fasciculus and the left acoustic radiation performed better on the QuickSIN. Further, morphometric results revealed more perceptually consistent listeners had reduced surface area and gray matter volume in left Broca's area. Behaviorally, we found QuickSIN scores were correlated with changes in the slope of listeners' identification curves suggesting a more stable categorization listening strategy yielded better (lower) SIN thresholds. DWI data

showed that listeners with greater white matter density in bilateral arcuate fasciculus and the left acoustic radiation performed better on the QuickSIN. Further, morphometric results revealed more perceptually consistent listeners had reduced surface area and gray matter volume in left Broca's area.

Conclusions: These findings support the notion that individual differences in phonetic categorization account for variance in SIN comprehension and are at least partially explained by differences in anatomical properties of the auditory-linguistic brain. Closer association between structures of the left cerebral hemisphere (surface area, gray matter volume) and behavioral response consistency in phoneme labeling suggests listeners with more stable phonetic perception make more efficient use of frontal language areas. Higher density in the acoustic radiations further suggests that the subcortical auditory pathways also contribute to better SIN understanding. This may involve afferent but also corticofugal efferent pathways.

SU60. Subcortical Speech Coding Predicts Perceptual Consistency in Phoneme Categorization and Improved Speech-in-Noise Perception

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Background: Listeners discretize the speech signal by assigning sounds to phonetic categories, though there is variability between individuals in how they accomplish categorization. Retaining more within-category acoustic information (gradiance) and having more consistent perception of sounds may be advantageous for understanding speech-in-noise (SIN). It is unclear how different levels of neural processing in the auditory system reflect these perceptual differences. In particular, whether subcortical speech-evoked responses drive perceptual consistency and gradiance in acoustic- mapping has not been thoroughly explored.

Methods: We recorded brainstem frequency-following responses (FFRs) while listeners actively labeled vowels along an acoustic-phonetic continuum using a visual analog scale. We measured individual differences in gradiance and consistency via phoneme labeling and SIN comprehension via the QuickSIN. We computed intertrial consistency of FFRs to index the stability of listeners' neural speech representation across stimulus presentations. We also assessed how faithfully midbrain responses represented stimulus acoustics using representational dissimilarity matrices (RDMs) computed across all token pairs. Neural RDMs were then compared with acoustic RDMs to estimate neuro-acoustic correlation (NAC) and thus whether FFRs carried gradient vs. discrete information of the speech signal.

Results: Greater behavioral consistency during phoneme labeling was correlated with improved SIN scores. Neurally, we found listeners with greater behavioral consistency also had more consistent subcortical representations of speech sounds in their FFRs. Likewise, more consistent listeners and more gradient listeners had higher NAC in subcortical RDMs, suggesting a more accurate and detailed encoding of the speech continuum.

Conclusions: Our findings reveal important benefits of perceptual consistency (and to a lesser extent gradiance) to several domains of speech perception. We find perceptual consistency is driven by more faithful stimulus feature encoding at a surprisingly early, subcortical level of the auditory system. More consistent sensory processing could provide a more stable readout of the

speech signal to higher cortical brain areas. This could in turn confer advantageous to later perceptual processes downstream, including understanding speech in noise.

SU61. Comparison of Speech in Noise Processing in Hearing Impaired Populations Using [15O]Water Pet

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Background: Difficulty with speech in noisy real-world environments remains a primary complaint in hearing impaired (HI) listeners despite successful use of amplification devices. Research in normal hearing listeners indicates that auditory cortex is active while abstracting speech objects from noise and provides input to fronto-temporal networks for further perceptual, attentional, and semantic analysis. We want to understand whether these are the same neural mechanisms across hearing impaired listeners with different devices (CI and hearing aid) and configurations, and how these mechanisms relate to successful hearing outcomes.

Previously we demonstrated in a proof-of-concept study that we could robustly and reliably measure brain [15O]Water positron emission tomography (PET) blood flow activity to speech in noise in cochlear implant users at a single subject level. We observed activity in a fronto-temporal network to speech in noise in each single subject. Here we expand our investigation to include a broad range of hearing impairment (CI and hearing aid users) and device configurations to capture variability that may be different to that of a matched control population.

Methods: We measured brain blood flow using [15O]Water PET in a group of 44 cochlear implant subjects, 20 hearing aid users, and 20 age-matched normal hearing controls while they performed a word-in-noise identification or noise-control task (matched on RMS sound level). Six runs were performed for each condition. On a given 3-min run for speech in noise (+15 dB SNR), word tokens were presented in multi-talker babble, and at random intervals subjects were prompted to perform a 4-alternative forced choice task. The control condition was a noise level detection task matched for response demands. PET data were analyzed in SPM12 using a flexible factorial model.

Results: We found robust activations in single subjects for the contrast speech in noise vs control noise in auditory cortex and inferior frontal cortex. Group level activation regions of interest in auditory cortex and inferior frontal cortex were significant, along with activations across a network of areas involved in language processing ($p < 0.05$, corrected). Group comparisons highlighted differences in the involvement of frontal cortex which may relate to the existence of acoustic hearing. A regression analysis including speech in noise task performance indicated the involvement of brain regions for attention and object analysis.

Conclusions: Our results show that speech in noise processing in different hearing impaired populations may depend on the availability of residual acoustic hearing, and the use of brain networks for language similar to that of the normal hearing population.

SU62. Intelligibility Modulates Early Attentional Filtering of Competing Speech

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Background: The intelligibility of competing speech influences how it interferes with perception of target speech. However, most previous studies have failed to equate local spectrotemporal statistics; thus, observed differences in performance may reflect differences in low-level acoustic features. Also, few measured underlying neural responses. Here, listeners performed a selective attention task, detecting target color words amidst competing, spectrotemporally matched intelligible or unintelligible maskers. We measured simultaneous electroencephalography (EEG) to gain insight into underlying mechanisms.

Methods: Twenty different normal-hearing listeners participated in each of two Experiments. Listeners were asked to attend to a target stream comprising color and object words spoken by a male talker, while ignoring a masker stream spoken by the same or a different male talker. Target words were randomly presented in time, while the masker stream was ongoing. The masker stream comprised either words drawn from the same set (Words masker), or a spectrotemporally scrambled version (Scrambled masker). Target and masker streams were also filtered in adjacent non-overlapping frequency bands to reduce energetic masking. Listeners were asked to detect target color words by pressing a button. We simultaneously measured evoked responses using EEG. In Experiment 1, the scrambling procedure resulted in a flat amplitude envelope masker. Experiment 2 served as a control, where the Scrambled masker stream was amplitude modulated with a Words masker envelope to match isochronous acoustic onsets.

We computed hit rates (responses to color words in the target stream), within-stream false alarm rates (responses to object words in the target stream), and between-stream false alarm rates (responses to color words in the masker stream in the Words condition). From the EEG, we computed the amplitude of onset sensory P1-N1 responses and P300 target recognition responses.

Results: Results replicated across experiments. A Scrambled masker yielded higher hit rates and lower within-stream false alarm rates than a Words masker. Participants performed better with a different talker than a same talker masker, but only with a Words masker. Between-stream false alarm rates with a same talker Words masker were significantly higher than with a different talker. Evoked responses mirrored behavioral performance. Onset (P1-N1) responses evoked by target color words were larger for a Scrambled masker than a Words masker. This effect was larger when the target and masker were spoken by the same talker. Target recognition (P300) was greater for target color than object words, but did not vary with masker type or talker.

Conclusions: Our results demonstrate that auditory attention is more strongly disrupted by intelligible speech than by unintelligible speech, even when their spectrotemporal properties, and thus the degree of energetic masking, are closely matched. Furthermore, intelligibility and talker differences were reflected in early sensory (P1–N1) responses, but not target recognition (P300) responses, implicating an early-latency attentional filtering mechanism.

SU63. Evaluation of Aided Speech Intelligibility, Listening Effort, and Subjective Sound Quality With Real-Time Deep Neural Network-Based Noise Reduction

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Background: Hearing loss affects many aspects of daily life, yet deficits in speech understanding are especially detrimental to professional productivity, social engagement, and quality of life. As a result, improvement in speech intelligibility, notably in noisy environments, is a primary goal of hearing aid design. Strategies to improve speech understanding include use of directional microphones, and signal processing to emphasize speech-content and reduce noise. Despite these innovations, hearing aid users still frequently report difficulty hearing speech in noise. Recent advancements in computational hardware and deep learning have now made it possible to include a real-time deep neural network (DNN) noise reduction system within the physical form factor of commercial hearing aids. Initial tests of the DNN noise reduction showed up to 10 dB SNR improvement for speech in noise based on acoustic measures (Raufer et al., 2024), and behavioral results show significant improvement in speech intelligibility and reduction in listening effort (Wright et al., 2024).

Methods: Here, to further evaluate this new technology, behavioral measures in the presence of speech-shaped noise tested speech intelligibility, listening effort, and subjective measures of sound quality for the DNN-capable device (with DNN-on and DNN-off), as well as comparable commercial devices from other manufacturers. Participants (N=29) had documented hearing loss and were experienced hearing aid users. The intelligibility task used materials adapted from the coordinate response matrix (CRM; Bolia et al., 2000), with sentences presented in the free field from a range of target-azimuth locations. Listening effort was measured using the adaptive categorical listening effort scaling task (ACALES; Krueger et al., 2017). The subjective listening task presented a 4-minute two-talker conversation, followed by a questionnaire assessing elements of speech clarity, naturalness, loudness, and separation from noise.

Results: Results from the CRM experiment showed significantly higher accuracy by listeners with the DNN-on device configuration compared to DNN-off, notably for target locations at the periphery (+/- 120°) with improvement in accuracy up to 30%. Participant ratings of listening effort were significantly lower for comparable SNRs (-2.9 dB) for the DNN-on configuration compared to DNN-off. Finally, subjective measures of speech quality were significantly better with the DNN for measures of clarity, naturalness, separation from noise, and overall satisfaction compared to alternate devices.

Conclusions: The DNN-capable device tested in this experiment represents the next technological advancement in the search for optimal listener performance by providing real-time noise reduction and subsequent improvement in speech understanding. Quantitative results consistently showed better performance with the DNN-on, as measured by speech intelligibility in the CRM task and reduced listening effort in the ACALES task, without compromising the subjective sound quality provided by the device. Future studies on device-performance in multi-talker environments and real-life listening situations will need to be done to further evaluate this new technology.

SU64. Effects of Listening Effort Demands on Cortical Tracking of Attended and Ignored Narrative Speech

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Background: Aging and hearing loss are associated with increased prevalence of speech perception difficulties in the presence of background noise. To maintain high comprehension performance in such challenging situations, listeners tend to exert greater amounts of cognitive resources, such as selective attention and context-based filling-in and predictive processes, which are collectively referred to as listening effort (LE).

While LE is increasingly recognized as an important factor in speech perception, it is relatively unknown how changes in LE manifest at the level of acoustic, linguistic, and semantic neural representations of speech. Multiple studies have previously reported that aging is associated with amplified cortical representations of continuous speech (e.g., Presacco et al., 2016; Decruy et al., 2019, Mesik et al., 2021), with increases in reliance on LE with age being one of the hypothesized causes of these changes. However, as LE was not directly manipulated in these studies, the extent to which cortical tracking of different speech features is affected by LE remains unclear.

Methods: In the present, ongoing study, we test whether varying the LE demands in a speech comprehension task modulates cortical processing of speech using a large sample of participants (target $n = 60$) spanning a wide age range (18-80 years). While undergoing EEG recordings, participants listen to pairs of audiobooks narrated by different male speakers, instructed to attend to one of the stories and ignore the other. On a trial-by-trial basis (i.e., every ~1 min of the story), LE demands are manipulated by pitch-shifting the ignored story such that the median F0 of the narrator either matches that of the target narrator, making them more difficult to distinguish (higher LE), or they differ by 4 semitones, making them easier to separate (lower LE). Following each trial, participants respond to several comprehension questions about the target story and rate their subjective LE.

Results: The EEG data from each LE condition are analyzed using temporal response function (TRF) analyses, which are used to decompose the EEG signal into contributions from the processing of attended and ignored speech at multiple levels of the processing hierarchy (acoustic, linguistic, and semantic). Effects of LE demands on cortical tracking of speech are evaluated by comparing the prediction accuracies of TRF models from each of the two conditions, and by directly comparing pairs of TRFs for the corresponding speech features between the LE conditions. The outcomes of these analyses will be used to explore how these TRF measures are related to age and cognitive status of the participants (working memory capacity, attentional inhibition, and fluid intelligence).

Conclusions: The results will help elucidate how recruitment of additional cognitive resources in effortful listening shapes cortical processing of speech at multiple levels of the speech processing hierarchy [Supported by NIH grant R21DC020788].

SU65. A Novel Algorithm for Estimating Psychometric Functions in the Matrix Speech-In-Noise Test With Automatic Speech Recognition

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Background: The matrix speech-in-noise test was first developed in Sweden in the early 1980s and is now available in more than 15 languages. Each sentence consists of five words with a fixed syntax: name, verb, number, adjective, and noun. Test material is organized into lists of 10 sentences, each containing unique words. The primary goal is to determine the signal-to-noise ratio (SNR) at which a listener achieves a predefined level of speech intelligibility, typically 50% of words correctly identified. Traditionally, two lists (20 sentences) are required to obtain this threshold. Here, we present a novel adaptive algorithm that uses all SNR–intelligibility data to estimate the full psychometric function. In addition, participant responses are scored automatically using speech recognition, allowing the test to be performed without a tester.

Methods: The procedure consists of three iterative phases. Phase 1 begins with an SNR where no words are recognized (0% intelligibility). The same sentence is then repeated with SNRs increased in 2 dB steps until 100% intelligibility is reached. All SNR–intelligibility pairs are stored, and a sigmoid psychometric function is fitted. From this curve, SNRs corresponding to 20%, 40%, 60%, and 80% intelligibility are extracted and used in Phase 2 with four new unique sentences. The resulting data are combined with Phase 1 to refine the psychometric function. Phase 3 repeats this procedure with updated SNRs and additional new sentences. The final psychometric function incorporates all data, enabling estimation of SNR thresholds at any intelligibility level.

Results: The new method was evaluated in 20 native Swedish speakers with normal hearing. Performance was compared between the classical algorithm (20 sentences, threshold at 50% intelligibility) and the new adaptive algorithm. The adaptive approach typically required fewer than 15 sentences to estimate the full psychometric function, compared with 20 in the classical method. Threshold SNRs at 50% intelligibility differed by < 1 dB between the two approaches. Automatic speech recognition, implemented with OpenAI’s Whisper, achieved nearly perfect accuracy in scoring participant responses.

Conclusions: The proposed adaptive scoring algorithm for the matrix test yields faster results while providing the entire psychometric function, rather than a single threshold value. The 50% SNR thresholds were nearly identical to those obtained with the classical method, confirming its validity. Automatic speech recognition proved highly reliable, enabling fully autonomous administration of the test without a human tester.

SU66. The Consistency of Spectral Contrast and Auditory Enhancement Effects at Higher Frequencies in Speech Perception

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Background: Perception of any sound is influenced by the spectrum of its preceding context. In spectral contrast effects (SCEs), a spectral peak in the preceding context stimulus promotes

perception of spectrally adjacent information in the target sound. In auditory enhancement effects (EEs), a spectral notch in the preceding context stimulus promotes perception of that same spectral region in the target sound (i.e. pop-out effect). SCEs and EEs have long been studied in isolation without much examination of a potential link between them. Stilp and Winn (2025 ARO) reported strong consistency in SCE and EE magnitudes affecting normal-hearing (NH) listeners' categorization of mid-frequency /d/-/g/ targets ($r = 0.76$). However, this consistency did not extend to perception of noise-vocoded speech ($r = 0.05$), possibly due to reduced access to fine spectral details distinguishing /d/ from /g/. Here we assessed the consistency of SCEs and EEs in wider-band, higher-frequency speech cues that distinguish /s/ (as in "seat") from /ʃ/ (as in "sheet"). We predicted the magnitudes of SCEs and EEs would be consistent in how they affected listeners' categorization of spectrally intact and degraded speech. We also predicted that spectral smearing would broaden spectral peaks (increasing SCEs) and make spectral notches less shallow (decreasing EEs).

Methods: Nineteen NH hearing listeners categorized target words from a 10-step continuum ranging from "seat" to "sheet". The target word was always preceded by a context sentence filtered to elicit SCEs and EEs. To elicit SCEs, a bandpass filter amplified energy from 2200-4500 Hz or 4500-8200 Hz to facilitate contrastive perception of "seat" and "sheet", respectively. A bandstop filter attenuated energy in these same frequency regions to elicit EEs.

Results: Both SCEs (more high-frequency "seat" responses when the context sentence had the lower-frequency range amplified) and EEs (more "seat" responses when the context sentence had the higher-frequency range attenuated) influenced perception of both spectrally intact and degraded speech. Data showed consistency among SCE and EE effect magnitudes in spectrally intact speech ($r = 0.45$) but not degraded speech ($r = 0.19$), showing that spectral degradation disrupts the symmetry between these auditory effects even when the target does not demand fine resolution. We observed larger SCEs than EEs in noise-vocoded speech ($p < .001$), consistent with predictions based on spectral smearing.

Conclusions: The consistency of EE and SCE magnitudes in NH listeners' speech perception extends to higher-frequency speech contrasts, but is disrupted with spectral degradation, indicating another fundamental aspect of hearing that is likely affected by CI processing.

SU67. Simulated Peripheral Adaptation Accounts for Spectral Context Effects in Speech Perception

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Background: Perception of any sound is influenced by the spectrum of its preceding context. In spectral contrast effects, a spectral peak in the preceding context stimulus promotes perception of spectrally adjacent information in the subsequent target sound. For example, when perceiving a sound that could be "da" or "ga", a precursor context sentence with prominent energy in low-F3 frequencies (1700-2700 Hz) encourages perception of the high-F3 target "da"; a context sentence with prominent energy in high-F3 frequencies (2700-3700 Hz) encourages perception of the low-F3 target "ga" (Stilp and Assgari, 2017). Here, we used computational modeling to evaluate the hypothesis that adaptation of auditory nerve fibers underlie these perceptual shifts.

Methods: Neural responses to stimuli from Stilp and Assgari (2017) were simulated using a model of the auditory nerve that takes peripheral nonlinearities into account (Zilany et al., (2014). Center frequencies of high-spontaneous-rate fibers spanned 2300-3000 Hz to cover F3 regions of interest. Average neural rates were calculated over a 50-ms window that began at the start of the formant transition. Then, rates were normalized by subtracting the mean of the population response to obtain the population-response shape. Following Maxwell et al. (2020), a decision variable was implemented for the model to mimic behavioral responses. On each trial, if the shape of the model's population response to the target syllable more closely resembled its response to the "da" endpoint of the target continuum (smaller Mahalanobis distance), the model selected a "da" response, and vice versa for "ga". This process was repeated 100 times to yield percent correct for each context sentence/target syllable pair.

Results: Simulated neural responses captured essential characteristics of listener data from Stilp and Assgari (2017). The model's "ga" responses monotonically increased as the frequency of F3 onset in the target syllable decreased (became more "ga"-like). Critically, neural responses showed evidence of a spectral contrast effect: more high-F3 "da" responses followed precursor sentences with amplified low-F3 frequencies, and more low-F3 "ga" responses followed sentences with amplified high-F3 frequencies.

Conclusions: Modeling results supported the hypothesis that neural adaptation in the auditory periphery can explain spectral contrast effects in speech perception. Shifts in simulated neural responses accounted for shifts in behavioral responses to the same stimuli. Future work will explore additional experimental paradigms and contributions by later stages of neural processing.

SU68. Subcortical Responses to Connected Speech: The Role of Language Experience

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Background: Prior studies have shown that both long-term language experience and short-term training can enhance subcortical auditory encoding of speech sounds. However, these investigations often rely on frequency-following responses elicited by repetitive, isolated speech stimuli (e.g., single syllables), which do not reflect the dynamic and continuous nature of everyday speech. The present study aims to examine the extent to which language experience influences subcortical processing of connected speech.

Methods: Participants included four groups of normal-hearing adults with varying levels of Mandarin Chinese proficiency: native English speakers with no exposure to tonal languages (n = 11), beginner-level Mandarin Chinese learners (n = 21), advanced Mandarin Chinese learners (n = 14), and native Mandarin Chinese speakers (n = 20). They listened to synthesized Mandarin sentences, each composed of four syllables, with an additional one to three syllables appended at the end of each trial. During the task, they engaged in cyclic syllable counting from 1 to 4 and reported the final count. To assess subcortical auditory processing, temporal response functions (TRFs) were used to model EEG responses using speech features estimated from auditory nerve models. TRF metrics—including model prediction accuracy, peak latency, and peak amplitude—were compared across groups to evaluate the influence of language experience on subcortical encoding of connected speech.

Results: TRFs across all groups showed a prominent peak around 8 ms, resembling the wave V of auditory brainstem responses, indicating subcortical involvement in processing connected speech. No significant group differences were observed in the latencies or amplitudes of this wave-V-like peak, nor in the model prediction accuracy.

Conclusions: These preliminary findings did not provide evidence for an effect of language experience on subcortical processing of connected speech. Further research is warranted to examine subcortical responses to more naturalistic, continuous speech stimuli.

SU69. Effect of Reverberation and Interruptions on Listening Effort

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Background: Everyday listening often occurs in complex environments that include distractions and distortions. Prior work on factors that disrupt spatial selective attention have been conducted in anechoic settings (Liang et al., 2023), but realistic settings involve reverberation that would affect the clarity of both the target signal and the distractors. Although reverberation increases difficulty of target perception, it could also reduce salience of distractors; furthermore, listeners can adapt to stable reverberation in the environment. The impact of reverberation on disrupted auditory attention is therefore unknown. This study tested whether reverberation amplifies or reduces the disruptive impact of interrupters on recall of a target, and whether pupil dilation can index these effects.

Methods: Participants (N=60, normal hearing) heard two competing, temporally interleaved streams of syllables with and without interruptions. Both streams consisted of syllables from the same male talker; they differed only in their location, with one stream spatialized to 30° left and the other to 30° right of center. All syllables and interrupters were spatialized using binaural room impulse responses (BRIRs).

At the start of each trial, a spatialized /ba/ cued the listener to the target stream. Streams comprised 4 syllables randomly drawn with replacement from [/ba/, /da/, /ga/]. Syllable onsets were 600 ms apart; the target began 300 ms before the distractor. At the end of the trial, subjects reported the target syllables in order. In 50% of trials, a novel interrupter occurred 125ms before the onset of the 2nd target syllable. Syllable recall accuracy was compared across conditions. The time course of pupil diameter was baseline-corrected, z-scored, and analyzed using cluster-based permutation tests; peak pupil size latency and amplitude were extracted as summary measures.

Results: Reverberation and interruption each significantly reduced syllable recall accuracy, with the strongest effects for syllables after the interrupter. Interrupters decreased accuracy by ~8%, and reverberation by ~6%. Pupil time courses showed significant clusters of increased dilation for interrupted trials in both environments. Peak amplitude analyses revealed larger dilation for interrupted versus uninterrupted trials, with a marginal effect of reverberation and a significant interaction: The reverberant, uninterrupted condition elicited the smallest peak amplitude despite

being behaviorally most difficult. Peak latency occurred earlier under both reverberation and interruption, with additive effects but no interaction.

Conclusions: Unexpected interrupters reliably increase cognitive effort, amplified under reverberation. Uninterrupted reverberation produced both the lowest accuracy and smallest pupil response, which may reflect reduced salience of syllable onsets and energetic masking from reverberant tails. These findings extend adaptation theories by demonstrating that although listeners may perceptually adjust to reverberation, working memory and attentional demands remain vulnerable to interference.

SU70. Optimized Feature Gains Explain and Predict Successes and Failures of Human Selective Listening

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Background: Attention enables communication in settings with multiple talkers, allowing us to select sources of interest based on their features. Decades of research have left two gaps in our understanding of feature-based attention. First, humans succeed at attentional selection in some conditions but fail in others, for reasons that remain unclear. Second, neurophysiology experiments implicate multiplicative gains in selective attention, but it remains unclear whether such gains are sufficient to account for real-world attention-driven behavior. To address these gaps, we optimized an artificial neural network with stimulus-computable feature-based gains for the task of recognizing a cued talker's speech, using binaural audio input (a "cocktail party" setting).

Methods: We optimized a deep neural network to report words spoken by a cued talker in a multi-source mixture. The model received an excerpt of the cued talker's speech in isolation, followed by a mixture of a different excerpt of the cued talker with other talkers and/or noise sources. Audio was spatialized within simulated reverberant rooms using human head-related transfer functions. Attentional gains were implemented via learnable logistic functions operating on the time-averaged model activations of the cue excerpt. The activations of the mixture were then multiplied by these gains. Gains could be high for features of the cue, and low for uncued features, as determined by parameters optimized to maximize correct recognition. Task performance was measured across a diverse set of diotic and spatial listening conditions for both models and human participants to quantify human-model behavioral alignment.

Results: The model successfully learned to use both spatial and vocal timbre cues to solve the word recognition task. Though not fit to human data, the model matched human performance, exhibiting selection based both on voice qualities and spatial location. The model also predicted novel attentional effects that we confirmed in human experiments. Finally, the model's internal representations exhibited signatures of "late selection" like those seen in human auditory cortex.

Conclusions: We provide a framework to quantitatively model feature-based auditory attention by optimizing a deep neural network to perform an attentional word recognition task. The results suggest that human-like attentional strategies naturally arise from optimization of feature gains for selective listening, offering a normative account of the mechanisms and limitations of auditory attention.

SU71. EEG-Based Neurofeedback Training of Auditory Selective Attention: Toward Clinical Translation Across Languages and Listening Conditions

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Background: Speech comprehension in noisy environments presents significant cognitive challenges, particularly for individuals with hearing impairments. While conventional hearing aids amplify sound, they often fail to improve listening in noisy settings, underscoring the importance of central auditory processing. Auditory selective attention modulates neural representations to enhance target signals over background noise, and individual variations in this ability correlate with speech-in-noise performance. Previous neurofeedback training (NFT), designed to enhance this attentional modulation, improved word-in-noise perception in normal-hearing English speakers. Building on this, the present work investigates two critical extensions for a step forward to a better clinical viability: 1) whether the training benefits generalize to speakers of a different language (Korean) and to more complex sentence-in-noise (SiN) tasks, and 2) whether the training is effective in a diotic condition, where the target and masker sounds are co-located, which is more representative of real-world listening and a crucial step for application in hearing-impaired populations.

Methods: Two groups of normal-hearing participants were recruited: ten Korean speakers and eight English speakers. All participants underwent weekly NFT sessions for three to four weeks. During training, participants attended to one of two simultaneous speech streams ("Up" with a female voice vs. "Down" with a male voice), presented either dichotically (to the Korean group) or diotically (to the English group). The target speech was visually indicated by coins placed above or below a center line. Based on the attentional state, decoded from single-trial EEG, a rocket icon moved up or down, either collecting or missing the coins. To evaluate the efficacy of training, the strength of attentional modulation and SiN perception were assessed before and after the training period. The strength of attentional modulation was measured by the magnitude of entrainment of the EEG to the attended vs. unattended speech envelope. SiN was assessed using sentences presented against multi-talker babble at varying signal-to-noise ratios (SNRs) and spatial conditions.

Results: Following the training, both studies demonstrated a trend of strengthened attentional modulation. A significant increase was observed in the magnitude of entrainment for the English-speaking group in the diotic condition (Cohen's $d=1.19$, $p=0.012$), with a similar positive trend noted for the Korean-speaking group (Cohen's $d=0.97$, $p=0.091$). Correspondingly, SiN performance showed trends of improvement when the two groups were combined (Cohen's $d=0.76$, $p=0.064$), particularly under challenging SNR conditions.

Conclusions: These complementary preliminary findings demonstrate the potential of this NFT paradigm to enhance sentence-based speech-in-noise perception across different languages and in a clinically relevant diotic listening condition. Further research involving larger populations, including individuals with hearing loss, will validate these results and advance the development of evidence-based rehabilitation strategies to improve communication in noisy environments.

SU72. Neural Correlates of One-Shot and Generalized Recognition Learning of Degraded Speech Signals

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Background: Separating neural correlates of conscious perception from sensory processing is a fundamental goal of cognitive neuroscience. Auditory stimuli that can help dissociate such neural correlates include degraded speech signals such as sine-wave speech (SWS) and noise-vocoded speech. Classic tasks using such stimuli often use "one-shot learning", utilizing the fact that while naive listeners hear SWS as noise or whistles, a single exposure to corresponding original speech (OS) can render SWS word-like. This permits the comparison of different percepts (before and after one-shot learning) despite identical stimuli, effectively separating stimulus processing from conscious perception. However, few studies have examined the neural plasticity associated with such learning or how such effects change across longer timescales (e.g. the course of a one-shot learning SWS experiment).

Methods: 19 participants listened to 60 pre-selected SWS tokens or phonetically similar foils, each taken from a previously curated dataset. Each subject was asked to listen to SWS both before and after hearing corresponding OS and in each case to select the word they perceived in a two-alternative forced-choice (target word or phonetically similar foil). Electroencephalography (EEG) was measured throughout and binned based on whether the word occurred before or after OS (i.e. pre- versus post-OS) and whether the word occurred early or late (i.e., in the first or second half of the experiment, respectively).

Results: Listeners were more accurate at recognizing target words after versus before OS. However, this effect was weakened as the experiment progressed, reflecting the fact that participants' accuracy on pre-OS SWS recognition also increased as the experiment progressed, suggesting generalized SWS learning. Slow cortical responses showed a one-shot learning effect at posterocentral electrodes after 500 ms as well as above-chance pre- versus post-OS decoding, particularly between ~200 and 600 ms post-stimulus. Reflecting generalized learning of SWS, this decoding effect weakened as the experiment progressed. However, this early-versus-late effect was only significant in the alpha band, consistent with time-frequency analysis showing significant early-versus-late differences in the alpha and beta bands from ~50-350 ms after stimulation.

Conclusions: Consistent with previous studies, our results show that listeners can both utilize immediately preceding context to help them recognize otherwise unintelligible SWS tokens, and that they can learn to perceive SWS more generally (i.e., without cues) on a relatively slower timescale, perhaps engaging a novel, learned listening mode for SWS. The neural plasticity underlying both effects is reflected in both the slow cortical potential and higher-frequency (e.g., alpha- and beta-band) EEG signals. Such longer-term learning effects should be considered in one-shot learning experiments.

SU73. Predicting Speech-In-Noise Performance and Noise-Reduction Outcomes With Electrophysiological and Subjective Measures

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Background: Individuals with similar clinical hearing profiles have varying tolerance to background noise, which is an inherent individual trait that may affect their reaction to noise-reduction (NR) processing commonly implemented in digital hearing aids. The present study evaluated such individual characteristics using electrophysiological measures and subjective noise-tolerance profiles to examine their relationship with speech-in-noise performance and NR outcomes.

Methods: The neural signal-to-noise ratio (SNR), an amplitude ratio between auditory evoked responses to target speech and noise onset, was calculated for each individual as a cortical index of noise tolerance from a cohort of 42 individuals with normal hearing. Further, noise-tolerance profiles were derived using k-means cluster analysis to assign participants into distinct subgroups based on their performance on a noise-tolerance domain test, which evaluates the relative importance of three criteria, including noise annoyance, speech interference, and listening effort, in judgment of noise tolerance.

Results: The neural SNR significantly correlated with speech-in-noise performance and NR outcomes with varying processing strengths. However, noise-tolerance profiles did not show any differences in such outcomes among different clusters of participants. The neural SNR and noise-tolerance profiles also did not show any meaningful relationships.

Conclusions: While the neural SNR showed a robust relationship with speech-in-noise performance and NR outcomes, and subjective noise-tolerance profiles were comparatively less sensitive, subjective measures remain one of the most accessible options in the clinic. Thus, we must continue to investigate ways to incorporate both electrophysiological and subjective measures to understand better individual characteristics that determine their noise tolerance levels and hearing outcomes.

SU74. Modeling the Effects of Temporal Incoherence Within Speech on Sentence Intelligibility

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Background: Temporal coherence—the synchronized timing of acoustic events across frequency channels—is considered a core cue in auditory scene analysis, supporting the perceptual grouping of components from a common source. While its importance has been demonstrated extensively with synthetic and non-speech stimuli, its role in speech perception remains unclear. In particular, introducing temporal incoherence into speech by desynchronizing frequency bands does not appear to make it harder to segregate a target speaker from a competing speaker than when listening to speech in steady noise (Lee and Oxenham, 2024, J. Acoust. Soc. Am. 156, 3285–3297). It is unknown how current computational models of speech intelligibility handle speech whose onset of the frequency bands are asynchronized, or whether their predictions would depend solely on the type of background masker. This study compared

model predictions to existing behavioral data to provide new insights into the relationship between model mechanisms and human performance.

Methods: We employed the sCASP model of speech intelligibility (Relaño-Iborra et al., 2019, J. Acoust. Soc. Am. 146, 3306–3317) to predict the behavioral results of Lee and Oxenham (2024). In that study, 25 normal-hearing listeners heard speech filtered into eight contiguous frequency bands and temporal coherence was disrupted by introducing delays between bands. The target speech was presented with either speech-shaped noise or a single competing talker as the masker. Both target and masker could be temporally coherent or incoherent, with the degree of incoherence varying across conditions. The sCASP simulated peripheral auditory processing and generated speech intelligibility predictions by correlating the modulation spectrograms of the clean target speech and the degraded stimulus.

Results: The model predicted a decrease in intelligibility as across-band delays increased, consistent with the general behavioral trend. However, sCASP overestimated the severity of this decline, particularly at larger delays. The model also predicted strong masker type effects, with greater degradation in the speech-in-speech condition. By contrast, human listeners showed relatively similar performance across masker types.

Conclusions: The study examined how temporal coherence affects intelligibility of speech in complex auditory environments, employing a well-established model of speech intelligibility (sCASP) to interpret behavioral data. Our preliminary results reveal mismatches between model predictions and human performance, highlighting limitations in current modeling approaches. These findings provide insights into the mechanisms underlying auditory scene analysis with speech and point to directions for further model development.

SU75. Neural Correlates of Spectro-Temporal Modulation Sensitivity

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Background: The ability to detect spectro-temporal modulation (STM) cues is closely linked to improved speech-in-noise perception, an ability particularly compromised in individuals with sensorineural hearing loss (SNHL). Impaired STM sensitivity has often been attributed to broadened cochlear tuning and is increasingly being considered a potential early indicator of SNHL.

A new clinical measure, the Audible Contrast Threshold (ACT), adaptively manipulates modulation depth to quantify STM sensitivity. This test has been validated across both normal-hearing and impaired listeners, showing promise as a practical and, importantly, language-independent tool for assessing auditory function. By avoiding linguistic confounds, ACT allows assessment in diverse populations and may support clinical decision-making regarding amplification strategies.

While behavioral studies highlight the real-world relevance of STM sensitivity, less is known about its neural correlates. Cortical responses to acoustic changes, measured as the Acoustic Change Complex (ACC), provide a physiologically grounded metric related to intelligibility. Using electroencephalography (EEG), we seek to characterize how cortical dynamics track STM changes and whether these signatures align with psychophysical markers such as ACT.

Methods: Human EEG recordings were collected while participants listened to STM blocks embedded in broadband noise. Stimulus design alternated between spectrally structured and unstructured sequences, with energy carefully equated across conditions. EEG was acquired using a 32-channel BioSemi system. Post-change neural features, including auditory steady-state responses (ASSR), band-limited power, root-mean-square amplitude, and global field power were analyzed. Logistic regression with stratified five-fold cross-validation was applied to decode modulation depth and classify clean versus noisy conditions. ACT scores were also obtained for all participants, serving as a complementary behavioral reference.

Results: Spectral decomposition revealed that the neural representation of STM stimuli varied across frequency bands. In particular, delta and theta phase coherence following stimulus change increased systematically with modulation depth, consistent with theories of neural entrainment in speech tracking. However, classification accuracy for modulation depth was marginally above chance, suggesting subtle cortical distinctions. In contrast, clean versus noise-added conditions were robustly classified, with area under the ROC curve well above chance, largely driven by ASSR contributions, suggesting that noise-related envelope coding elicits stronger cortical signatures than modulation depth encoding.

Conclusions: Our findings suggest distinct cortical signatures of STM encoding in the human brain, aligning neural entrainment with behavioral measures of modulation sensitivity. By combining EEG with ACT, this work bridges psychophysical and neural levels of analysis, offering a stronger framework for assessing auditory deficits in SNHL. Parallel EEG studies are ongoing using several pre-clinical chinchilla models of SNHL to garner more direct mechanistic insight. Overall, these cross-species insights further support the translational use of STM measures for precision clinical diagnostics and intervention planning.

SU76. External Validation of an Acoustic Change Complex Prediction Model for Speech Perception in Adults With Sensorineural Hearing Loss

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Background: The acoustic change complex (ACC) is a cortical neural response to changes in an ongoing sound, recorded via electroencephalography. Previous work by our group (Vonck et al., 2022) presented an ACC-based prediction model to predict the behavioral speech reception threshold (SRT), which explained 87% of the variance in SRT in normal-hearing and hearing-impaired individuals. This model suggests that the ACC could serve as an objective tool for assessing auditory performance, especially in populations with limited language or cognitive abilities. Our research aims to externally validate the ACC-based prediction model in a broader population with varying degrees of sensorineural hearing loss (SNHL).

Methods: Eighty adults aged 18-65 years (35 men, 45 women) with varying degrees of SNHL participated in this study. ACCs were evoked using acoustic change stimuli consisting of a 3000 ms reference tone at 1000, 2000, or 4000 Hz, gliding to a 300 ms target tone with a 12% higher frequency (i.e. 1000-1120 Hz, 2000-2240 Hz, 4000-4480 Hz). Each participant underwent pure-tone audiometry to determine normal hearing or the degree of SNHL. The SRT was obtained

using the Leuven Intelligibility Sentences Test (LIST). To assess test-retest reliability of the ACC, 52 participants completed a second ACC recording 1-4 weeks after the initial session.

Our aim is to validate the ACC prediction model by Vonck et al. (2022): $SRT = -6.4 + 0.071 \cdot HL + 0.083 \cdot (ACC_{latency} - 100)$, with HL being the pure-tone average at 500, 1000, 2000, and 4000 Hz and ACClatency the average latency of the three ACC recordings.

Results: While the Spearman correlation between predicted and behavioral SRT was strong ($\rho = 0.750$, $p < 0.001$), Bland-Altman analysis revealed poor agreement with increasing discrepancies at higher mean values. Intraclass correlation further confirmed limited reliability of the model across the full range of hearing loss ($ICC = 0.266$, $p = 0.004$). However, ACC test-retest reliability was excellent ($\rho = 0.834$, $ICC = 0.952$, both $p < 0.001$).

Conclusions: External validation revealed that the current ACC prediction model performs adequately only in individuals with normal hearing to mild hearing loss. To generalize across broader SNHL profiles, model refinement is necessary. Further refinement of the model is currently ongoing to improve its applicability. Nonetheless, the high test-retest reliability of the ACC supports its potential as a robust objective measure.

SU77. Estimating Speech Recognition Reference Curves Using Audiometric Measurements

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Background: In clinical practice, audiometric tests—including tone and speech audiometry—are essential for assessing hearing acuity. Tone audiometry measures the softest perceptible sounds per frequency, establishing individual hearing thresholds, while speech audiometry evaluates word recognition across sound levels, producing a speech recognition curve. Comparing an individual's curve to a normal-hearing reference provides insight into speech intelligibility loss and helps optimize hearing aid settings.

Proper calibration is critical for reliable measurements. ANSI and ISO standards define procedures and allowable deviations, enabling consistency across equipment and locations. However, unlike pure-tone audiometry, broadband speech calibration lacks standardized methods. Previous studies have explored different procedures, but changing audiometers or transducers can still affect speech recognition outcomes, even with standard calibration. For example, improved intelligibility of 4.6 dB was reported for Sennheiser HDA200 headphones compared to free-field expectations.

Differences between free-field and headphone listening arise from anatomical acoustic effects and technical characteristics of transducers (e.g., Telephonics TDH39 vs. Sennheiser HDA200). Establishing new reference curves requires many participants and is resource-intensive, while multiple curves increase error risk. This study aims to identify and quantify these differences and develop a predictor to estimate reference curves from technical parameters, eliminating the need for repeated subject testing in the future.

Methods: Thirty-two native Dutch-speaking adults (18–25 years) with normal hearing participated. Eligibility was confirmed via history taking, pure-tone audiometry, bone conduction thresholds, and tympanometry. Audiometric testing was conducted in a sound-insulated room

using an Otometrics Astera2 audiometer under five conditions: free-field (Yamaha MP5S loudspeaker), DD450 headphones, and TDH39 headphones, both with and without audiometer-applied frequency filtering. Calibration followed ISO and IEC standards, and inverse filtering was applied to create unfiltered test material. Speech stimuli consisted of 30 Dutch word lists (Bosman and Smoorenburg, 1995). Speech recognition was assessed monaurally in the better ear, starting at 30–40 dB SPL and decreasing in 5 dB steps over six lists per condition. SRTs and slopes were derived by fitting a Gaussian function using maximum likelihood optimization.

Results: Despite careful calibration and comparable pure-tone thresholds, the DD450 consistently yielded higher speech recognition scores than the TDH39. These differences persisted across filtered and unfiltered conditions, indicating that headphone choice significantly influences speech intelligibility. Although variations in the headphones' frequency response may partly account for these discrepancies, the response characteristics alone do not fully explain the observed effects. Revisions to the calibration procedures in current standards may therefore be recommended to ensure that speech recognition results are independent of the transducer used.

Conclusions: Even when calibration standards are met and hearing thresholds are equivalent, headphone selection can alter measured speech recognition. These findings highlight the necessity of transducer-specific reference curves and motivate the development of predictive models for speech recognition thresholds, enabling reliable clinical assessments across varying audiometric setups.

SU78. The Effect of Spectral Degradation, Level, and Noise on Discourse Comprehension and Listening Effort

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Background: Speech is often masked or distorted by background noise, with communication in background noise often occurring at increased sound levels. Speech perception often decreases at these higher levels, in a phenomenon known as “Rollover.” Hearing loss increases communication difficulty through decreased recognition of single words to impacts of comprehension of longer passages of discourse. One of the best available treatments for moderate-to-profound hearing loss is the cochlear implant (CI), but CI users struggle to communicate in noisy environments. Vcoded speech is used to simulate reduced spectral resolutions experienced by CI users. We investigated communication difficulties in noise under conditions that typically produce rollover, then measured discourse comprehension and listening effort in young normal-hearing adults when they listened to clear unprocessed or vocoded speech under quiet or background noisy conditions at different stimulus levels. We hypothesized that behavioral performance will be maximum in unprocessed speech but will significantly decline at 85 dB SPL in unprocessed and vocoded noisy speech, with this decline followed by increased listening effort at higher sound levels under noisy conditions.

Methods: Nine young normal-hearing adults between the ages of 18-30 years with no neurological or visual impairments listened to 1-1.5-minute-long literary passages, either unprocessed or vocoded. Passages were presented over headphones at sound levels of 35, 55, 65, 75, and 85 dB SPL, in quiet and in background noise at a +10-dB SNR. While listening,

participants focused on a red cross displayed at the center of a screen so an eye tracker could record their pupil dilation as a measure of listening effort. After each passage, participants answered comprehension questions to assess how well they understood the material.

Results: Behavioral performance decreased at the highest sound levels (85 dB SPL) for both unprocessed and vocoded speech in noise, which is consistent with the presence of rollover. Pupillometry revealed that listening effort peaked during unprocessed speech at 75 dB SPL in noise, suggesting that even with non-vocoded acoustic signals, increased intensity in noisy conditions forced subjects to exert more effort while listening to passages. For vocoded speech in noise, pupil dilation was lower than that of unprocessed speech at 75 dB SPL and steadily declined, possibly indicating participants' disengagement with increased task difficulty.

Conclusions: Findings suggest rollover is present during speech in noise but is not correlated with increased listening effort. In young normal-hearing adults, listening effort remained consistent across unprocessed and degraded speech, with no significant relationship with rollover as performance decreased. Additionally, listening effort peaked at a lower level than behavioral performance, indicating that listeners can compensate for a degraded, louder signal with increased effort to maintain behavioral performance. For future research applications, we would maintain challenging auditory conditions while exploring how listening effort is impacted by time-of-day effects.

SU79. Tuning In: Effects of Low-Frequency Allocation and Fine-Structure Timing on Pitch Perception in Cochlear Implant Users

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Background: Conventional cochlear implant (CI) coding strategies, such as the Advanced Combination Encoder (ACE), primarily transmit slow-varying temporal envelope cues while discarding acoustic temporal fine structure (TFS). Although effective for speech in quiet, this limits access to pitch-relevant cues—especially in the low-frequency range crucial for music perception and tonal language comprehension. This study examines whether enhancing low-frequency access and/or reintroducing TFS timing can improve pitch sensitivity in CI users. Using a within-subject psychophysical design, we compare pitch perception across four coding strategies that systematically vary low-frequency allocation and TFS encoding.

Methods: Adult CI users (data collection ongoing) are tested using the Cochlear NIC research interface, which bypasses clinical processors. Four coding strategies are evaluated: (1) ACE-NMT: Baseline ACE implementation from Cochlear's Nucleus MATLAB Toolbox, (2) ACE-LF: Custom ACE variant with expanded low-frequency channel allocation to improve access to fundamental frequency (F0) cues,

(3) HD-ACE: High-definition ACE variant (similar to HDCIS) with increased pulse density to support TFS encoding, and (4) Peak-Derived Timing (PDT): Novel strategy that introduces low-frequency-biased timing by placing pulses at waveform peaks.

Results: All strategies are loudness-balanced, with consistent mapping parameters where possible. Each participant completes three psychophysical protocols, presented in randomized order: (1) Electrode Psychophysics: Discrimination of place, rate, and combined cues to assess

baseline pitch sensitivity, (2) Pure-Tone Pitch Perception: Frequency-difference limens (FDLs) at 110, 220, and 440 Hz, and (3) Musical Pitch Perception: F0-difference limens (F0DLs) for piano tones matched in fundamental frequency.

Stimuli are processed through each coding strategy. Thresholds are estimated using adaptive procedures targeting 75% correct. Linear mixed-effects models (with subject as a random factor) evaluate strategy effects. Secondary analyses assess whether individual baseline sensitivity predicts benefit from enhanced coding.

Conclusions: We hypothesize that ACE-LF will improve FDLs at low frequencies (110–220 Hz) relative to ACE-NMT; HD-ACE and PDT will yield additional improvements by reintroducing TFS timing, especially at lower F0s; and that individual benefits will correlate with sensitivity to place and/or rate cues.

This study provides a systematic, psychophysically grounded comparison of conventional and novel CI coding strategies using both synthetic and ecologically valid stimuli. Findings will guide future CI sound coding aimed at enhancing pitch perception in music and complex listening environments. Preliminary results will be presented.

SU80. The Elusive Bilingual Inhibitory Control Advantage and Its Role in Suprathreshold Hearing: An Investigation

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Background: As multilingual populations grow, it is important to understand how bilingualism influences systems that support speech perception in noise (SPIN). Selective attention and working memory are two cognitive processes that are crucial for filtering task-irrelevant sounds in noisy environments. However, the effect of multiple language exposures on these cognitive processes is still unclear. Here, we hypothesized that bilinguals would outperform monolinguals on auditory tasks that require distraction suppression but show comparable performance on tasks with minimal cognitive demands.

Methods: We recruited 153 participants (ages 18-40) through the online platform Prolific - 65 individuals whose first language (L1) is English and 88 others who are bilingual in English (L1) and Spanish (L2). All participants were right-handed, living in the U.S. or Canada, and had normal hearing. They completed four tasks designed to measure different cognitive abilities related to speech perception and working memory. The matrix sentences task evaluated informational masking by testing participants' ability to filter through multiple speakers while focusing on a target speaker. The auditory Stroop task assessed distraction suppression by having participants respond to prompts while ignoring conflicting auditory information. The Modified Rhyme Task assessed energetic masking by having participants identify a target word masked by inharmonic tones. The n-back test measured working memory by requiring participants to recognize stimuli presented earlier in a sequence.

Results: Bilingual participants showed a decreased Stroop cost, suggesting a bilingual advantage in distraction suppression. However, no group differences were observed in working memory, or the informational masking task. Surprisingly, monolinguals outperformed bilinguals in the energetic masking task, indicating that bilingualism may come with a greater cost of lexical recovery when retrieving words presented without linguistic context. Overall, findings support a conditional bilingual advantage confined to tasks with inhibitory control demands.

Conclusions: Bilingual listeners showed enhanced distraction suppression and inhibitory control. Ongoing analyses are focused on examining duration and degree of bilingualism in explaining variance on SPIN performance. Future work will test additional language pairings, broader age ranges, and real-world outcomes (e.g., listening effort, fatigue, device use) to identify when bilingual experience confers benefit, or cost, for everyday listening. Clinically, this condition-specific bilingual advantage has implications for personalized counseling and diagnostic testing.

SU81. Differential Effects of Masker Type on Speech-In-Noise Perception in ADHD

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Background: Attention Deficit Hyperactivity Disorder (ADHD) is associated with impairments in working memory and cognitive control, which are critical for goal-directed sensory behavior. These deficits may make it difficult for individuals with ADHD to selectively attend to speech in complex acoustic environments. Prior work suggests adults with ADHD experience increased cognitive load and expend more effort than neurotypical peers during speech perception-in-noise (SPIN) tasks. This effort is likely tied to limitations in working memory and attentional control, which are necessary for separating target speech from competing sounds. We hypothesize that individuals with ADHD will show lower accuracy on a SPIN task with informational masking compared to controls. We further expect that working memory and distraction suppression ability will predict SPIN task performance.

Methods: This study compared SPIN processing in individuals with and without ADHD in energetic and informational masking conditions. The Modified Rhyme Test with an inharmonic tone complex served as an energetic masker, while the Matrix Sentences task was paired with a non-overlapping informational masker to probe auditory processing at higher levels. The signal-to-noise ratio (SNR) between target and masker was varied to determine thresholds. Working memory was assessed via an n-back task ($n = 1-3$). Distraction suppression was measured using an Auditory Stroop task, calculating Stroop cost as the difference in reaction times between incongruent and congruent trials.

Results: Participants with ADHD demonstrated significantly higher SNR thresholds on the informational masking task relative to neurotypical peers, but no group difference emerged for the energetic masking condition. Initial group comparisons of distraction suppression via Stroop cost were not significant; however, after controlling for age, gender, and baseline reaction time, the ADHD group performed significantly worse. No group differences were found in working memory as measured by the n-back task.

Conclusions: The observed SPIN deficit under informational, but not energetic, masking indicates that ADHD-related deficits in speech perception occur beyond the cochlear level. The significant Stroop cost difference supports the role of reduced distraction suppression in explaining poorer SPIN outcomes for ADHD individuals. These results suggest attentional control, rather than initial auditory encoding, underlies the group differences in SPIN performance. Ongoing analyses will include questionnaire data on self-reported ADHD symptoms and medication use, allowing exploration of relationships between symptom severity, medication, and SPIN perception.

SU82. Cross-Species Physiological Measures of Spectrotemporal Modulation Processing

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Background: Restoration of speech audibility through amplification is a cornerstone of current audiological practice. Yet, individuals with sensorineural hearing loss (SNHL) often struggle to understand speech, even when it is audible, particularly in noisy environments. Psychophysical measures of sensitivity to spectro-temporal modulations (STM) have emerged as language-independent assays of suprathreshold auditory fidelity that can reliably predict speech-in-noise performance. However, behavioral testing is impractical in infants and other difficult-to-test groups and the neural correlates of this predictive power remain unknown. The acoustic change complex (ACC), an EEG response to modulation onset, may provide an objective neural correlate of STM sensitivity. This project seeks to establish normative baselines and optimized testing parameters for the STM-based ACC by collecting data in both normal-hearing humans and chinchillas. These foundational measures will then provide the framework for subsequent application across the continuum of normal hearing and SNHL in both human listeners and preclinical chinchilla models.

Methods: Human participants undergo otoscopy and full audiometric evaluation to confirm normal hearing. Behavioral measures include word identification in noise using the Modified Rhyme Test (MRT) materials, and STM sensitivity for noise carriers as in Zaar et al., (2023; 2024). Electroencephalography-based (EEG-based) ACCs were acquired as neural metrics of STM processing. Stimuli for ACC measurements were 2 seconds long consisting of a 1-s unmodulated reference segment smoothly transitioning to a 1-s spectrotemporally modulated segment. Responses to both tonal and noise carriers were collected. ACC responses are analyzed for reference-to-STM transition as a function of modulation depth and will be compared directly to behavioral STM thresholds in human listeners. Analogous stimuli are also employed for chinchillas with ACC responses measured using both subdermal electrodes and a 32-channel mini-EEG cap. Specifically, because peripheral frequency tuning in chinchillas is known to be substantially broader than psychophysically measured frequency selectivity in human listeners (Temchin et al., 2008; Shera et al., 2010), measurements in chinchillas span a wider range of STM parameters.

Results: Preliminary data show that ACC responses are measurable in both species. In human listeners, preliminary data also show that behavioral STM thresholds are predictive of speech-in-noise outcomes, replicating the work by Zaar et al. (2023; 2024)

Conclusions: These results highlight the feasibility of using the ACC as a candidate neural marker of suprathreshold auditory processing relevant for speech-in-perception. Data collection in both species is ongoing with the goal of expanding the sample. Human ACC measures will be compared to behavioral STM measurement to evaluate the translational potential of this approach to populations and settings where behavioral testing is not feasible. Chinchilla ACC norms established through this work will be used in future studies in SNHL chinchilla models.

SU83. The Long-Term Impact of Childhood Mild Traumatic Brain Injuries on Adult Auditory Processing Abilities

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Background: Mild traumatic brain injury (mTBI), or concussion, is highly prevalent in children yet long-term neural effects are still unclear. While many children appear to recover, prior studies suggest lasting deficits in auditory working memory, speech-in-noise perception, and increased listening effort that can persist into adulthood. Normal aging independently contributes to similar declines in auditory processing abilities causing everyday communication challenges. However, the interaction between early-life concussion and age-related auditory decline remains largely unexplored, leaving a critical gap in our understanding of long-term outcomes due to mTBI across the lifespan.

Methods: 252 North American English-speaking adults, aged 18-72, were recruited via an online platform, Prolific. All participants reported normal hearing, and passed a preliminary headphone check and hearing screening. A total of 104 participants self-reported a history of childhood mTBI, while 148 served as age- and sex-matched controls. Participants completed a concussion history questionnaire, post-concussion symptom survey, and the Hearing Handicap Inventory for Adults (HHIA). Auditory assessments included speech-in-noise perception (Matrix Sentences), auditory working memory (N-back), selective attention and inhibitory control (Auditory Stroop), and energetic masking (Modified Rhyme Test).

Results: Adults with childhood mTBI performed significantly worse in a speech-in-noise task emphasizing informational masking, while no differences were observed in energetic masking conditions. Additionally, a childhood history of mTBI was also significantly associated with decreases in auditory working memory assessed using an n-back task, and inhibitory control assessed using Auditory Stroop. While aging and mTBI each negatively impacted performance on the inhibitory control and the informational masking task, mTBI did not amplify age-related declines across the lifespan, suggesting the effects of concussion are long-lasting but largely independent of aging.

Conclusions: Childhood mTBI has persistent negative effects on auditory processing in adulthood, particularly in tasks requiring working memory, inhibitory control, and involving informational masking. These deficits appear independent of aging and separate from energetic masking, underscoring the long-term impact of pediatric concussion. Clinically, results emphasize the importance of considering concussion history when evaluating adults with speech-in-noise complaints despite normal audiometric thresholds, as these patients may present with central rather than peripheral deficits. Ongoing analyses will examine the role of injury

frequency, age of occurrence, and subjective hearing handicap in shaping outcomes. Together, this work highlights the need for proactive monitoring, patient counseling, and development of rehabilitation strategies to reduce the enduring consequences of childhood mTBI.

SU84. Using Mobile EEG for Tracking Auditory Attention in Realistic Scenarios: Introducing Attention Switches and Conversations

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Background: Auditory attention decoding (AAD) identifies which sound source a listener focuses on in multi-talker settings. This is key for future hearing aids (HAs), where neural signals could guide selective amplification of attended speech. Generally, there has been a heavy focus within AAD to use controlled lab setups with clean, audio-only stimuli and record brain activity via stationary equipment such as magnetoencephalography (MEG) or wired electroencephalography (EEG). While effective, these lack ecological validity, as real-world listening involves attention switching, visual cues, and eye gaze changes.

Recent work moves toward realistic scenarios with more complex tasks (e.g., switching attention, audiovisual stimuli), and mobile devices such as ear-centered EEG arrays (cEEGrids) and wireless EEG caps are important steps toward future brain-controlled HAs. Here, we recorded mobile EEG from 24 normal-hearing participants in a novel audiovisual (AV) multi-talker paradigm with three AV conditions: sustained attention, switching attention, and attending to a podcast conversation.

Methods: The participant sat in a sound-attenuated room with three monitors at azimuths 0 degrees and 30 degrees left/right, and four loudspeakers at 30 and 90 degrees left/right. Two AV stimulus sets were used: (1) single talkers speaking to the camera and (2) a two-person podcast conversation. Conditions included sustained attention, switching between talkers, and attending either the AV conversation or a side audio-only speaker.

EEG was recorded with a 44-channel scalp cap and 20-channel cEEGrids, eye movements with a static tracker, and sound with two ceiling microphones. Linear temporal response functions (TRFs) were applied forward (predicting EEG from speech) and backward (reconstructing speech from EEG). Analyses included TRF peaks (P1, N1, P2), reconstruction correlations, optimal lags, and classification, for comparison between speech features, attention, EEG setups, audio sources, and conversation settings.

Results: Scalp EEG revealed significant attention-related P2 peak differences between attended and ignored speech. cEEGrid data showed weaker correlations, highlighting the need to investigate optimal preprocessing settings further. Performance did not differ between sustained and switching conditions, suggesting robustness to attention shifts. Classification accuracy ranged from 55–70% across decision windows (1.1–35 s), supporting feasibility in varied listening tasks with mobile EEG.

Conclusions: This study extends mobile EEG beyond single-talker audio-only settings to AV sustained attention, switching, and conversations. While cEEGrids showed weaker attention modulation, scalp EEG provided robust results with clear TRF signatures and stable model performance. Backward models generalized across tasks, with classifiers exceeding chance in all conditions. These findings bring neural tracking closer to realistic applications and future brain-controlled HAs.

SU85. Speech Perception in Quiet and Noisy Environments in Mandarin-Speaking Cochlear Implant Users With Low Frequency Residual Hearing

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Background: The residual hearing (RH) is important for the rehabilitation results after cochlear implant (CI) surgery.

Methods: Thirty-seven sensorineural hearing loss (SNHL) patients (41 ears) with different degree of low frequency residual hearing (LFRH) underwent CI at our department from June 2017 to July 2024 were enrolled. Speech recognition included the monosyllable and disyllabic word in quiet, the sentence in quiet, the consonant, vowel, and lexical tone in quiet, and sentence in noisy environment. Correlations between preoperative LFRH and improvement in postoperative speech recognition rate after surgery were analyzed.

Results: Patients enrolled included six ears implanted with SY28, five with F28, six with MS, two with 422, 10 ears 512, two with 622, five with CA, two with SJ, and two with 522 electrodes. The mean age at CI surgery was 18.9 years (range: 4-44 years). The mean threshold of 125, 250, and 500Hz was 52.0dB (5-80dB), 59.9dB (35-90dB), and 73.7dB (30-105dB) HL, respectively. The preoperative recognition rate was 16.5% in monosyllable, 18.0% in disyllabic, 27.8% in sentence in quiet. The postoperative (mean, 7.6 months) rates were 65.8% in monosyllable, 73.0% in disyllabic word, 85.5% in sentence in quiet, 64.8% in consonant, 67.3% in vowel, 84.0% in lexical tone, and 4.1dB signal noise rate (SNR) in sentence in noise. The speech recognition was significantly improved at nearly one year after CI surgery ($p < 0.05$). The preoperative threshold of 125 Hz was significantly correlated with the improvement of disyllabic word recognition post CI ($p=0.0382$, $r=0.1056$).

Conclusions: Patients with residual hearing experience a significant improvement in speech recognition approximately one year after CI, and the preoperative 125Hz threshold is correlated with the postoperative improvement in disyllabic word recognition.

SU86. Frontal Cortex Modulation of Frequency Tuning During Word Recognition in Noise in Middle-Age and Older Adults

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Background: Difficulties in understanding speech in noisy environments present a significant challenge for many older adults. Pre-stimulus frontal activity appears to support word recognition in noise, including for older adults with hearing losses. One potential mechanism for

this performance benefit is frontal modulation of auditory cortex, a hypothesis supported by evidence that inhibition of cingulate cortex diminishes auditory discrimination in animals. We tested the hypothesis that pre-stimulus frontal cortex activity modulates or alters frequency tuning in auditory cortex during a word recognition in noise task and examined the extent to which tuning effects interact with age-related hearing loss.

Methods: A retrospective analysis was performed with data from 45 middle-aged and older participants (average age = 64.7 ± 7.3 years) who had normal hearing to moderate hearing loss. Participants were asked to identify words presented in speech-shaped noise (speech: 88 dB SPL, noise: 73 dB SPL; +15 dB signal-to-noise ratio). The words and noise were presented in the relative quiet between sparsely collected functional magnetic resonance imaging (fMRI) data. Spectral power in one-third-octave bands was computed for each word. For each band, a GLM was fitted that modelled words on trials with higher vs. lower pre-stimulus frontal-cortex activity, as well as a parameter for spectral power in that band. Voxel-wise characteristic frequency (CF) in auditory cortex was then defined by the band with the strongest BOLD association. Frequency tuning was examined based on the strength of BOLD associations with spectral power for CF and non-CF bands. The strength of these associations was compared between high- and low-frontal activity trials.

Results: Elevated pre-stimulus frontal cortex activity was significantly associated with higher auditory cortex BOLD contrast for both CFs and non-CFs, across subjects and speech frequencies [paired $t(224) = 3.13$, $p = 0.002$]. Participants with more hearing loss also appeared to show smaller auditory cortex responses to spectral power for specific one-third octave bands. For example, elevated 0.5 kHz pure-tone thresholds were associated with significantly smaller increases in CF effects for trials that were preceded by high pre-stimulus frontal activity [$r(31) = -0.39$, $p = 0.02$].

Conclusions: The results suggest that frontal cortex modulates auditory cortex frequency tuning during word recognition in noise by increasing the responsiveness of auditory cortex to variation in spectral power, but to a lesser extent of listeners with more hearing loss. Together, these findings are consistent with frontal modulation of auditory cortex during word recognition in noisy conditions.

SU87. Analyzing Deep Learning and Classical Machine Learning Methods for Classifying Communication and Head Movement Behaviors in Live Multi-Participant Conversations

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Background: Head movements play a pivotal role while engaged in multi-talker conversation by providing non-verbal feedback to partners and enhancing a listener's ability to separate sound sources. Commercial hearing-aids with an on-board IMU (Inertial Measurement Unit, i.e., accelerometers) typically use that information for step-counting and activity levels. At least one device uses it as an input to their environment classifier and integrated directional microphone. None, however, use the IMU to detect specific patterns of head movements to predict behaviors such as nodding, head shaking, listening to a person versus a video, or talking. Training an

automatic classifier to accurately detect these behaviors first requires collecting head movement data during multi-person conversation and laboriously annotating each behavior-type for each participant with high temporal precision. From that point, with the goal of training the most accurate model and integrating with the hardware in the hearing aid to improve device performance, the question is how best to model the data. To address this gap, we collected accelerometer data during natural multi-person conversations and paired it with detailed human annotations of communication and head-movement behaviors.

Methods: Head-movement data was collected from three cohorts of young, normal-hearing individuals (three per cohort) in a controlled, conference-room setting during 50-minute multi-talker conversations. Participants wore hearing aids with on-board accelerometers, and audio-video was recorded for each talker. Videos were manually annotated for communication and head-movement behaviors, including conversational turns and nonverbal cues such as tilts and nods. Temporal and spectral features were extracted from the accelerometer data (windowed into 1-second segments) corresponding to roll and pitch movements. These features, combined with the annotated behaviors, were used to train and test machine learning models. Models were trained on data from all but one participant and then tested on the held-out participant, repeating this procedure across all individuals.

Results: Several deep learning and classical machine learning models were compared for classifying communication behaviors (e.g., talking, listening, watching video) and head orientations (e.g., turning left or right, facing down, facing forward). More specifically, various sequence-to-sequence models, a state-of-the-art deep learning technique, were utilized. These models incorporated modern architectural components such as transformer networks. Multiple performance metrics were used to evaluate models, and results suggest that modern deep learning models outperform classical machine learning methods by significant margins. Classification performance improved further when temporal sequence information was incorporated.

Conclusions: These results indicate that during multi-talker conversation, hearing-aid accelerometers can automatically classify stereotypical behaviors with high temporal resolution (1-second). Even when tested on unseen subjects, the models remained reliable. This establishes a foundation for more advanced approaches that combine behavioral and movement patterns, further integrate temporal dynamics, and incorporate additional inputs to improve accuracy and ecological validity.

SU88. Cortical Response Differences in Hearing-Impaired and Normal-Hearing Older Adults During Speech Perception in Noise

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Background: At the neural level, auditory streams develop along a hierarchical pathway: primitive acoustic features are encoded as early as the auditory brainstem and continually refined up to the primary auditory cortex; from there, perceptual-feature representation and ultimately object and category-specific representations are formed along the ventral cortical pathway. To investigate how selective attention operates within this hierarchical pathway, we conducted an experiment using electroencephalography (EEG). Our previous studies with younger and older

listeners have shown prominent late-latency cortical responses to changes in spatial location for steady-state noise and speech targets. The present experiment specifically investigated how hearing impairment in older listeners affects these late-latency cortical responses.

Methods: Older, hearing-impaired adults ($n = 20$) and older, normal-hearing adults ($n = 18$) were tested in the free field. Listeners were presented with a speech stream either in quiet or in background babble at 6-dB signal-to-noise ratio (SNR). Speech materials were taken from a corpus of ~2500 monosyllabic words spoken by a male voice with an interstimulus interval of 100 ms, and babble consisted of non-English conversations from 10 talkers. Listeners either passively listened or responded by button press after the speech stream changed location among 5 possible locations in the front hemifield. Cortical responses were measured using a 64-channel EEG system. EEG preprocessing was performed using the Brainstorm analysis software, followed by custom signal processing and statistical analysis in MATLAB.

Results: The EEG global field power (GFP) was characterized by N1, N2, and P3 deflections. Notably, all components were observed in both active and passive tasks, consistent with prior findings in younger normal-hearing listeners. Preliminary results suggest that participants' P3 responses to speech occurred earlier in passive conditions compared with active attention ones. During passive listening, P3 activity was higher in normal-hearing subjects than those with hearing impairment; this difference was not observed during the later P3 peaks in the active attention tasks. Earlier components (N1 and P2) were also significantly larger in normal-hearing individuals, particularly during passive conditions. Additionally, hearing impairment was associated with differences in P3 amplitude across specific spatial angles.

Conclusions: Shorter latencies could reflect primarily bottom-up, stimulus-driven processes, whereas later responses might be associated with top-down, task-related processing. The fact that the prominence of these components diverge across the listening groups could be indicative of poor audibility, as well as possible compensatory effects. Future work may include effects of intervention (e.g., amplification) on emphasizing bottom-up processing effects in the hearing-impaired listener group

SU89. Central Encoding Deficits and Compensatory Behavioral Strategies After Peripheral Deafferentation in Guinea Pigs

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Background: Exposure to moderate-to-intense noise can cause temporary threshold shifts and permanently decreased auditory brainstem response (ABR) wave I amplitudes. These decreases are thought to arise from the peripheral deafferentation (PD) of high-threshold auditory nerve fibers that encode sounds in loud listening conditions. However, the extent to which decreased ABR wave I amplitudes correlate with speech-in-noise perception deficits has been a subject of much debate, with recent studies yielding conflicting results. Resolving this question is critical for understanding the neural underpinnings of 'hidden' hearing loss and developing effective diagnostic and therapeutic strategies.

Methods: To help resolve this question, we developed a guinea pig model to investigate PD effects on a complex sound recognition task (vocalization-in-noise categorization). We induced

PD with noise exposure and confirmed that ABR wave I amplitudes were permanently decreased while thresholds recovered to ~5 dB of baseline.

Results: We found that local field potentials (LFPs) in the auditory cortex were altered after PD, indicating changes in central sound encoding. We expected this combination of peripheral and central deficits to significantly affect categorization behavior. Surprisingly, however, animals continued to perform well in the categorization task. Behavioral analyses revealed that the animals adopted different compensatory strategies, with a high degree of individual variability, to overcome the underlying encoding deficits. To determine whether the high performance after PD could be the result of engaging top-down mechanisms, we used pupillometry in passive listening animals to measure behavioral correlates of largely bottom-up processing. We found that vocalization-in-noise categorization thresholds were indeed affected at loud sound levels, consistent with high-threshold fiber loss.

Conclusions: These preliminary results suggest that top-down mechanisms can powerfully mask PD-induced deficits present in the ascending auditory pathway. We propose that taking these central compensatory mechanisms into account is essential for resolving the debate on the relationship between PD and speech perception deficits.

SU90. Dose-Dependent Ototoxicity Effects of Gentamicin and Ethacrynic Acid in a Chinchilla Model of Snhl

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Background: In preclinical models, sensorineural hearing loss can be induced by targeting specific cochlear structures using controlled noise exposure or ototoxic drugs. In chinchillas, carboplatin exposure selectively targets inner hair cells. A mild noise exposure protocol causes reversible threshold elevations but permanent synaptic loss between auditory- nerve fibers and IHCs (cochlear synaptopathy), whereas a moderate noise exposure protocol produces permanent damage to IHCs, outer hair cells, and synapses. Additionally, previous studies have shown that high combined but single doses of ethacrynic acid and gentamicin lead to near-complete OHC destruction, though these reports have focused primarily on anatomical and histological outcomes. The goal of this research is to develop a dosing strategy that induces gentamicin-related OHC-based hearing loss with threshold shifts like those produced by the moderate noise exposure protocol, while preserving IHCs, and to investigate the associated physiological changes in cochlear function. Optimizing efficient gentamicin protocol will provide a reliable model for studying OHC-specific contributions to SNHL and for evaluating therapeutic strategies.

Methods: Two approaches were used to induce OHC damage in chinchillas. In the first, gentamicin was administered intramuscularly in combination with intravenous ethacrynic acid, which has been used in the past to achieve OHC damage with single doses. In the second, animals received only repeated systemic gentamicin dosing over several. Hearing function was evaluated at baseline and at multiple post-treatment time points using auditory brainstem responses, middle ear muscle reflexes, and otoacoustic emissions, including distortion-product, stimulus-frequency, and transient-evoked measurements.

Results: Gentamicin combined with ethacrynic acid produced dose-dependent effects on cochlear function in chinchillas. While lower doses caused only subtle increases in ABR thresholds and small reductions in OAEs, higher doses resulted in moderate-severe to severe threshold shifts and near-complete loss of OAEs, consistent with at least widespread OHC destruction. However, the extent of this damage exceeded what typically observed in the moderate noise exposure protocol, which produce more moderate threshold elevations and preserve some OAEs, suggesting only partial OHC dysfunction. The higher gentamicin dose with ethacrynic acid was also associated with systemic health complications. Together, these findings suggest that although gentamicin with ethacrynic acid reliably induces OHC-related hearing loss, its severity can surpass the moderate noise exposure model and highlights the need to optimize dosing to achieve moderate threshold shifts while preserving IHCs, which we are continuing to pursue.

Conclusions: These preliminary findings underscore the importance of carefully optimizing gentamicin–ethacrynic acid dosing strategies to selectively damage OHCs without affecting IHCs. Establishing such a protocol will provide a consistent and efficient experimental model of OHC-specific SNHL. Beyond its research value, this model will contribute to the development of more precise diagnostic approaches that can eventually be translated to clinical practice, improving the identification of cochlear pathologies, and guiding more effective therapeutic interventions.

SU91. Relationship Between Distorted Tonotopic Coding and Suprathreshold Spectrotemporal Modulation Perception in Sensorineural Hearing Loss

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Background: Threshold audiometry and restoration of speech audibility through amplification are cornerstones of current audiological practice. Yet, individuals with sensorineural hearing loss (SNHL) often struggle to understand speech, even when it is audible, particularly in noisy environments. Psychophysical measures of sensitivity to spectro-temporal modulations (STM) have emerged as language-independent assays of suprathreshold auditory fidelity that can reliably predict speech-in-noise performance. However, the mechanistic basis underlying individual differences in STM sensitivity among patients with SNHL remains unknown. Emerging cross-species evidence from our team and others suggests that SNHL not only broadens auditory filter "tips," as traditionally recognized, but also distorts the fundamental tonotopic organization of the cochlea. Specifically, temporal responses of the cochlear base can be commandeered by "off-frequency" (lower frequency) sound fluctuations. Furthermore, chinchilla models reveal that the degree of distorted tonotopy varies according to the specific cochlear pathology underlying SNHL. For instance, suprathreshold tonotopy remains largely intact in metabolic hearing loss but is significantly distorted in noise-induced hearing loss, which is associated with outer hair cell damage. Consistent with animal models, human studies demonstrate that individuals with similar clinical hearing profiles can exhibit widely varying tuning-curve tip-to-tail ratios (TTRs) and associated degrees of distorted tonotopy. These individual variations in distorted tonotopy are a candidate mechanism to explain why patients with similar audiograms can differ markedly in their STM sensitivity and speech-in-noise

comprehension. This study directly tests whether individual variations in markers of distorted tonotopy predict individual differences in STM sensitivity and speech-in-noise performance.

Methods: To investigate the relationship between distorted tonotopy and STM sensitivity, we recruited individuals along the continuum of normal hearing to symmetric moderate SNHL (N=32 thus far) to participate in lab measurements to gather STM thresholds, speech-in-noise (SPIN) thresholds, and psychophysical tuning curves. Separately, anonymous participants (N=53 thus far) were recruited to participate in online web-based measurements where STM thresholds, SPIN thresholds, and tuning-curve TTRs.

Results: Linear modeling analysis of in-lab data revealed that TTRs, a correlate of distorted tonotopy, are predictive of aided SPIN scores, and online data replicated this association. Additionally, online STM thresholds predict SPIN replicating the results from Zaar et. al (2024). In turn, STM thresholds were predicted by TTRs.

Conclusions: These preliminary data showing associations between TTRs, STM sensitivity and SPIN scores are consistent with the notion that distorted tonotopy is a prominent contributor to suprathreshold hearing outcomes in SNHL. Further data collection is ongoing.

SU92. Fidelity and Validity of the French-Canadian Version of the Communication and Acceptance Scale (FR-CAS)

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Background: Hearing loss can significantly affect various aspects of daily life. Constantly needing to ask others to repeat themselves or having to explain one's hearing difficulties and coping strategies can lead to feelings of fatigue, frustration, or discouragement. Over the years, numerous questionnaires have been validated to assess the functional impacts of hearing loss. However, hearing care professionals in French-speaking regions of Canada have access to very few tools that have been validated in their language. The Communication and Acceptance Scale (Öberg and al., 2020) is a Swedish questionnaire that comprises 22 items, including 18 statements rated on a Likert scale ranging from "totally agree" (1 point) to "totally disagree" (5 points). This study aimed to 1) translate and culturally adapt the Communication and Acceptance Scale (FR-CAS), and 2) evaluate the metrological qualities of its French-Canadian version.

Methods: This study was conducted in two phases: the first involved cross-translation, and the second focused on validation. For the first phase, the questionnaire underwent a rigorous double-translation process, accompanied by consultations with French-speaking and Swedish-speaking individuals with knowledge in the field of communication disorder, as well as audiologists, to ensure linguistic, conceptual and cultural equivalence. For the second phase of the study, a total of 90 participants were recruited. All were aged 18 or older, fluent in French, and self-identified as living with hearing loss. Participants were asked to complete three instruments: the (FR-CAS), the Hearing Handicap Inventory for the Elderly- Screening version (HHIE-S), and the Échelle de mesure de difficultés d'audition (ÉMDA). To assess test-retest reliability, participants completed

the FR-CAS a second time following a two-week interval and intraclass correlation analysis was conducted. A Cronbach alpha was measured to assess internal consistency and Spearman correlations were conducted to assess concurrent validity between the questionnaires.

Results: The internal consistency of the overall scale was strong, with a Cronbach's alpha of 0.81. Test-retest reliability was also robust ($r = 0.810$, $p < 0.001$), indicating good temporal stability. Concurrent validity was moderate, but statistically significant, when comparing total scores from the FR-CAS with both the HHIE-S ($r = -0.682$, $p < 0.001$) and the EMDA ($r = -0.615$, $p < 0.001$). All correlations were similar to those reported for the original Swedish CAS.

Conclusions: The French version of the Communication and Acceptance Scale (FR-CAS) demonstrated strong validity and reliability, establishing it as a new tool for hearing professionals in French-speaking regions of Canada. It offers a meaningful way to assess individuals' acceptance of hearing loss and its effects on communication and emotional well-being.

SU93. Longitudinal Changes in Acoustic Absorbance in Cochlear Implant Recipients

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Background: Patients often experience decrements in acoustic hearing sensitivity following cochlear implantation.

When hearing recovers, conductive hearing loss is suspected (Chole et al., 2014). When hearing loss persists, this is often attributed to cochlear trauma. Some post-surgical changes impacting sound transmission are transient (Racca et al., 2022), though permanent ones have also been suggested (Scheperle and Hajicek, 2020). Cochlear conductive hearing loss may also contribute (Banakis Hartl et al., 2016). Correctly identifying the mechanism(s) responsible for post-operative hearing loss is critical to improving hearing preservation outcomes and to identifying the most appropriate medical and audiological management for the individual patient.

This study focuses on evaluating middle ear sound transmission using longitudinal absorbance measurements in newly implanted CI recipients. The aims include (1) establishing the incidence of suspected conductive hearing loss, (2) describing the timeline and frequency characteristics of recovery, and (3) characterizing permanent changes to middle ear transmission. Individual variability is of interest as changes post implantation may arise from multiple factors impacting different frequency regions.

Methods: Fifteen cochlear implant (CI) recipients have participated to date with various lengths of follow-up post-surgery. Video otoscopy and wideband acoustic immittance measures are being performed serially: at surgery (prior to implantation) and at each post-operative visit (initial activation, 0.5-, 1-, 3-, 6-, and 12-months post activation.) Otoscopic images are evaluated post-hoc for evidence of hemotympanum, effusion, or other notable findings. Baseline absorbance measurements are compared to published normative data, and post-operative absorbance is compared to the subject's own pre-operative baseline.

Results: Pre-operative measurements were not always normal. Some ears had a history of previous ear surgery (e.g. myringotomy tubes) or external ear abnormalities (e.g. exostosis). No ear was excluded; each subject's pre-operative measurements served as their baseline for comparison with their post-operative results

Evidence of hemotympanum/effusion was seen frequently at initial activation (~2 weeks post-surgery). Absorbance for ears with abnormal otoscopic impressions will be compared with absorbance for ears with normal otoscopic impressions. Effusion was visually observed up to 2 months. The frequency patterns, direction, and magnitude of absorbance changes relative to pre-operative baselines were highly variable across subjects. Within a subject, the absorbance systematically changed over time, though resolution to baseline was observed in only a few cases. These patterns will be quantified further as more data are available.

Conclusions: Sound transmission through the outer and middle ear is impacted by cochlear implantation. The effects are observed immediately post-surgery and change over time. Otoscopy can help identify changes to the middle ear system visible through the tympanic membrane, while absorbance patterns provide insight into changes that are less visible and may persist beyond the acute healing phase.

SU94. A Multi-Biomarker Battery for Stratifying Complex Sensorineural Hearing Loss and Predicting Listening Outcomes

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Background: Complex sensorineural hearing loss (SNHL) can involve multiple components of the peripheral and central auditory pathways, but standard clinical measures of hearing like the audiogram are primarily sensitive to OHC dysfunction. Other pathologies, such as cochlear synaptopathy or inner-hair-cell dysfunction, can remain undetected despite their detrimental effects on neural coding of sound and perception. These hidden pathologies, with or without co-occurring OHC dysfunction, are likely to contribute to variability in perception and treatment outcomes. Non-invasive biomarkers that detect the diversity of cochlear pathologies potentially present in SNHL are needed to better understand and address the complaints of individuals with hearing difficulties. Pre-clinical animal models of SNHL provide mechanistic insight into the effects of specific pathology profiles on physiological biomarkers, and studies in humans improve our understanding of the perceptual consequences of hearing loss. In this cross-species study, we use a battery of biomarkers in both human and animal subjects to dissect the clinical category of SNHL, expanding previous work to investigate multiple subtypes of hidden and overt hearing loss.

Methods: Pre-clinical chinchilla models of four types of sensorineural injury were used to assess the sensitivity of each biomarker to isolated and co-occurring pathologies, induced either with noise or ototoxic drugs. Before and after exposure, animals completed a physiological battery including auditory brainstem response (ABR) thresholds, ABR to a high-level click, envelope following response (EFR), otoacoustic emissions (OAEs), and the middle ear muscle reflex (MEMR). Human participants with normal hearing or mild to moderate SNHL were also tested

with the same physiological measures. In addition, human subjects completed three speech-in-noise tasks to relate physiological measures of hearing to speech perception.

Results: In chinchillas, biomarkers showed changes after exposure in at least one group, but no single biomarker was sufficient to accurately classify animals by their exposure group. Classification accuracy significantly increased when a combination of biomarkers was used. The most accurate model included a combination of distortion product OAEs, MEMR strength, phase-locking value, pure-tone average, EFR magnitude, and ABR Wave I amplitudes. In human subjects, including physiological measures improved the identification of individuals with poor speech-in-noise performance. Specifically, MEMR and stimulus frequency OAEs were sufficient for identifying the poorest performing individuals with normal hearing thresholds.

Conclusions: This work highlights the critical need to consider biomarkers beyond the audiogram for diagnosing hearing loss. A combination of measures sensitive to both OHC and non-OHC dysfunctions is needed to improve the diagnostic specificity of SNHL. Additionally, a large, standardized dataset will be necessary for more detailed profiling that takes advantage of advances in machine learning based classification techniques. To effectively analyze the contributions of these factors to hearing impairment, a multi-metric physiological, cross-species profiling approach will be essential.

SU95. Predicting Profiles of Cochlear Pathology From Non-Invasive Physiological Biomarkers

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Background: Temporal-bone histology shows that damage to outer hair cells, inner hair cells, the stria vascularis, and the auditory nerve contribute to sensorineural hearing loss.

Physiological measures such as otoacoustic emissions, the middle ear muscle reflex, and the auditory brainstem response are used both in research and clinically to detect these pathologies and to aid in identifying the site of lesion. However, it remains difficult to establish a one-to-one correlation between physiological responses and histological profiles, especially when more than one pathology is present (as is typically the case with complex sensorineural hearing loss). In this study, we examined the relationship between individual histological profiles of cochlear pathology in several pre-clinical chinchilla models and responses on a battery of physiological tests sensitive to both OHC and non-OHC dysfunctions. Our goal is to identify individual profiles of sensorineural hearing loss through physiological biomarkers of cochlear pathology.

Methods: Chinchillas with and without experimentally induced hearing loss (noise or ototoxic drugs) were used. Distortion product otoacoustic emissions (DPOAEs), stimulus frequency otoacoustic emissions (SFOAEs), wideband middle ear muscle reflex (MEMR), auditory brainstem responses (ABR), and envelope following responses (EFR) to a rectangular amplitude modulated tone were collected on each animal. Pre- and post-treatment data were collected for exposed animals. After all testing was completed, animals were euthanized and perfused. Cochleas were harvested, post-fixed, and decalcified. Cochleas were then bisected through the modiolus, immunolabeled, and dissected into half turns. Specimens were labeled for hair cells (Myo7a), afferent neurons (NFH), hair cell presynaptic ribbons (CtBP2), and cholinergic efferent

neurons (ChAT). In a subset, stereocilia were also labelled (ESPN). Confocal z-stack images were obtained at .5, 1, 2, 4, and 8 kHz cochlear locations.

Results: The noise-exposure protocol resulted in a mild to moderate elevation of ABR thresholds and reduced DPOAE amplitudes. Most of these animals, however, showed intact hair cells at the tested cochlear locations. Gentamicin exposed animals showed profound elevation of ABR thresholds and complete loss of hair cells. Consistent with prior findings, hair-cell counts alone underestimated the effects on auditory physiology. Many animals with overt elevations in hearing sensitivity showed little to no loss of hair cells. Further image analysis will also quantify the contribution of stereocilia dysfunction to responses on physiological assessments in the absence of hair cell loss.

Conclusions: Profiling individual degrees of pathology across multiple components of the cochlea is critical to understanding the contribution of various pathologies to hearing and is necessary for precision diagnostics and ultimately pharmaceutical treatments for hearing loss. Hair-cell survival is not enough to explain the effect of noise on physiological assessments of hearing. These findings highlight the need for more nuanced understanding of the relationship between histopathology and physiology.

SU96. Auditory Neuropathy Associated with Kinase Suppressor of RAS2 Deficiency

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Background: The kinase suppressor of RAS 2 (KSR2) plays a critical role in regulating energy intake, energy expenditure, and substrate utilization. In humans, mutations in the KSR2 locus are associated with severe early-onset obesity and insulin resistance. Studies in Ksr2-knockout (KO) mice demonstrate that Ksr2 expression and function in the hypothalamus are centrally regulated, leading to neuroinflammation, hyperphagia, altered metabolic rates, and consequently obesity and type 2 diabetes (T2D). Among the many mouse genes linked to obesity, Ksr2 deletion produces one of the most profound obese phenotypes, often resulting in early lethality. Obesity, in turn, is inconveniently associated with an elevated risk of sensorineural hearing loss (SNHL), with metabolically unhealthy obesity conferring an even greater risk. A recent meta-analysis confirmed that obesity correlates with a higher prevalence of hearing impairment in adults. In this study, we evaluated auditory function in Ksr2-deficient mice to determine whether obesity driven by Ksr2 deletion contributes to the development of SNHL.

Methods: Ksr2-KO mice on a C57BL/6J-Tyrc-Brd x 129SvEvBrd hybrid background were originally obtained from Lexicon Pharmaceuticals (Woodlands, TX) and transferred to the Veteran's Affairs Loma Linda Healthcare System (VALLHS). The colony was maintained using a heterozygous × heterozygous breeding strategy and housed in a modified Specific Pathogen Free (SPF) facility, without sound conditioning of ambient noise, with ad libitum access to food and water. After weaning, mice were provided a standard diet (Teklad #7001, Envigo) and weighed weekly, while breeders were maintained on a higher-energy diet (Teklad #8626). At approximately 20 weeks of age, hearing function in littermate mice of both genotypes, Ksr2-KO (Ksr2^{-/-}) and wild-type (Ksr2^{+/+}), was assessed using auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE).

Results: As they entered adulthood, Ksr2-KO mice rapidly developed obesity, whereas sex-matched littermates gained weight more gradually and often reached a plateau. By 20 weeks of age, the body weight of Ksr2-KO mice was nearly double that of their littermate controls. DPOAE responses showed deterioration at high-frequency regions with substantial inter-animal variability; however, overall responses were comparable between Ksr2-KO and wild-type mice. Similarly, ABR thresholds did not differ significantly between genotypes. Notably, wave-I ABR amplitudes were markedly reduced in Ksr2-KO mice for both click ($p < 0.0001$) and tonal stimuli ($p < 0.001$, two-way ANOVA).

Conclusions: Although Ksr2-KO mice exhibited a severe obese phenotype, their DPOAE responses and ABR thresholds remained normal, indicating preserved outer hair cell function. In contrast, the significant reduction in wave-I ABR amplitude suggests impaired synaptic transmission between inner hair cells and spiral ganglion neurons, consistent with classic auditory neuropathy. Similar to peripheral neuropathy—which is common in obesity- and T2D-associated neuropathies and typically affects the hands and feet—auditory neuropathy also represents a prevalent form of neuropathy in obesity and T2D populations.

SU97. Sparse Labeling of Spiral Ganglion Neurons Reveals Differences in Spike Initiation Zone Distances by Synaptic Position

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Background: Cochlear hair cells transduce auditory stimuli and send this information to the central nervous system through spiral ganglion neurons (SGNs). The dendrites of type I SGNs are typically unbranched and contact a single inner hair cell. The position of the synapse along the inner hair cell is correlated with synaptic morphology, sound intensity sensitivity, transcriptional profile, and the ion channel properties of post-synaptic type I SGNs. However, the extent to which diversity in SGN output reflects contributions from presynaptic hair cell machinery, intrinsic neuronal properties, or dendritic attenuation remains unclear. To address the latter, we measured the distance between synaptic terminals and spike initiation zones and will present a preliminary model that integrates these measurements with our prior ion channel characterizations as a foundation for a whole-neuron model of Type I SGNs.

Methods: To identify the spike-initiation zone and dendritic length on individual neurons, SGNs were sparsely labeled through two viral transfection strategies. First, an AAV that drives the expression of Cre recombinase was delivered intracerebroventrically in postnatal day 1-3 (P1-3) Ai14 (floxed tdTomato) mice at doses that drive sparse neuronal expression of tdTomato. Second, to selectively label modiolar-contacting SGNs, a Cre-dependent GFP virus was injected into the posterior semicircular canals of P1–P3 NF107Cre mice. Four weeks after injection, cochleae from both ears were collected and immunostained for confocal imaging of fluorescent dendrites and Caspr, a paranodal structural protein marking spike initiation zones.

Results: Across 83 modiolar-contacting and 46 pillar-contacting type I SGNs, we found that modiolar-contacting neurons had significantly shorter dendrite lengths from the peripheral terminal to the Caspr-labeled spike initiation zone. On average, modiolar-contacting dendrites were around 20% shorter than their pillar-contacting counterparts. These findings were repeated

in mice where GFP was expressed only in NF107Cre-positive neurons. 59 out of 68 NF107Cre; GFP positive neurons synapsed on the modiolar side of the inner hair cell. Consistent with these neurons being ‘modiolar’ contacting, these results closely match the proportion of Calretinin-negative NF107Cre; Ai14 somata in the spiral ganglion (223 out of 242).

Conclusions: Whether a neuron successfully fires an action potential depends on many factors, including the expression of ion channels and dendritic morphology. Our goals are to disentangle how anatomical and biophysical factors jointly determine the functional output of SGNs. These results highlight sparse labeling and the NF107Cre line as valuable tools for tracking dendritic lengths and selectively targeting modiolar-contacting type I SGNs. Ongoing work will integrate these data with previous biophysical characterizations of ion channels to assess the relative contributions of dendritic morphology and ion channel properties to the cellular mechanisms underlying SGN subtype diversity.

SU98. Aberrant JAK2 Activation During Development Alters Auditory Thresholds, Neural Gain, and Postsynaptic Architecture

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Background: The JAK-STAT pathway is a central regulator of cytokine and growth factor signaling, with well-established roles in hematopoiesis and inflammation. However, its role in auditory development remains largely unexplored. Aberrant JAK2 signaling is known to drive hematologic disorders and alter immune cell function, raising the possibility that it may also influence cochlear maturation and neural encoding. We investigated how constitutive activation of JAK2 (Jak2CA/WT Pax2Cre) impacts auditory function, outer hair cell (OHC) physiology, spiral ganglion neuron (SGN) subtypes, and synaptic organization.

Methods: Jak2CA/WT Pax2Cre⁺ (EXP) and Jak2CA/WT Pax2Cre⁻ (CTRL) mice were assessed at P28-30. Auditory brainstem responses (ABRs) measured thresholds, P1 latency, intensity, and P1-N1/P3-N3 amplitude intensity functions across 4 - 64 kHz. Distortion product otoacoustic emissions (DPOAEs) were used to evaluate outer hair cell function. Cochlear histology included spiral ganglion neuron (SGN) subtype labeling (Tuj1, Calb1, Calb2, Peripherin), synaptic ribbon - postsynaptic pairing (CtBP2/PSD95), and afferent innervation (neurofilament, myosin 7A).

Results: EXP mice exhibited elevated ABR thresholds across the frequency spectrum, indicating a broad deficit in auditory sensitivity. Latency intensity analysis revealed significant conduction delays at 16 and 32 kHz. Although P1-N1 amplitudes were preserved, higher-order P3-N3 amplitudes were reduced at 5.6-32 kHz, suggesting impaired central auditory gain. DPOAEs demonstrated elevated thresholds and diminished growth functions, particularly at mid-to-high frequencies, consistent with compromised OHC function. SGN analysis showed a significant reduction in Calb2-positive neurons in the apical turn, while Tuj1-, Calb1-, and Peripherin-positive subtypes were preserved, pointing to selective vulnerability of a neuronal population important for temporal encoding. Synaptic ribbon counts and CtBP2/PSD95 pairing were largely intact, but PSD95 puncta appeared globular and disorganized in EXP mice, suggesting altered postsynaptic maturation rather than frank synapse loss. Afferent fiber density and gross

innervation patterns were preserved, supporting the conclusion that functional deficits arise from subtle postsynaptic and neuronal subtype changes rather than widespread neurodegeneration.

Conclusions: Constitutive JAK2 activation disrupts early auditory development at multiple levels. Physiologically, EXP mice show global threshold elevation, conduction delays, impaired OHC function, and reduced higher-order amplitude growth, reflecting deficits in both peripheral sensitivity and central gain. Structurally, these deficits are accompanied by selective SGN subtype loss and altered postsynaptic organization, despite preservation of overall ribbon counts and afferent innervation. These findings identify JAK2 as a novel regulator of cochlear development and neural encoding. Importantly, they suggest that JAK2-targeted therapies, now widely used in hematologic disease, may have unanticipated consequences for auditory function.

SU99. Angiotensin Receptors as Targets for Protection From Noise-Induced Hearing Loss

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Background: The renin–angiotensin–aldosterone system is a ubiquitous endocrine system with significant contributions to inner ear physiology. In opposition to angiotensin II binding to its type 1 receptor (AT1R), which drives inflammation and fibrosis, binding of the type 2 receptor (AT2R) promotes vasodilation, anti-fibrotic, and neuroprotective effects. We hypothesized that AT2R antagonism would impair recovery of auditory function after noise-induced hearing damage, and that conversely, AT1R antagonism using losartan would protect against hearing loss.

Methods: Thirty-two mice (16 male, 16 female) were randomized into four groups of 8. AT1R antagonism was achieved with losartan-infused chow, while AT2R antagonist PD123319 was delivered via continuous subcutaneous infusion via osmotic mini-pump. Controls received standard chow and/or subcutaneous saline. Groups were as follows:

- A: AT1R and AT2R antagonism (losartan + subcutaneous PD123319)
- B: AT1R antagonism (losartan + subcutaneous saline)
- C: AT2R antagonism (control chow + subcutaneous PD123319)
- D: Control chow and subcutaneous saline

Surgical subcutaneous placement of Alzet osmotic mini pumps was performed 1 day prior to noise exposure, and losartan treatment was initiated immediately following noise exposure. Auditory brainstem responses (ABR) were measured at 8, 16, and 32 kHz from 90–10 dB in 5 dB steps at baseline and at 1-, 7-, 14-, and 30-days following noise exposure (8-16kHz octave band noise, 114dB SPL for males, 116dB SPL for females). Experimenters were blinded to group assignment. One-tailed Mann–Whitney U tests were performed to compare elevated post-noise ABR thresholds of select groups.

Results: As compared to baseline levels, noise overexposure resulted in permanently elevated ABR thresholds in all groups, as expected. By day 7, Group C (AT2R inhibitor drug alone) exhibited significantly worsened thresholds at 8 kHz compared with controls in Group D (median 85.0 vs 55.0 dB, $p = 0.0105$), with persistent differences at day 14 (82.5 vs 55.0 dB, $p =$

0.0177) and day 30 (75.0 vs 50.0 dB, $p = 0.01235$). In contrast, AT1R inhibition in Group A (Losartan plus AT2R inhibition) compared to Group C (AT2R inhibition alone) attenuated the noise-induced ABR shift in a protective manner at 8, 16, and 32 kHz across all time points after day 1, except for 8 kHz at day 30 and 32 kHz at day 14. For all comparisons, the magnitude of differences was diminished at higher frequencies.

Conclusions: Type 1 and Type 2 angiotensin receptors confer opposing effects in our mouse model of noise-induced hearing loss. Availability of AT2Rs for activation may provide endogenous protection against worse hearing outcomes during acoustic trauma. In the absence of AT2R signaling, pharmacologic AT1R inhibition with losartan results in significant levels of protection against severe noise toxicity. AT1R inhibition and AT2R activation may represent overlapping strategies for achieving hearing protection against loud noise.

SU100. Hair-Cell Degeneration in Hipsc-Derived Inner-Ear Organoids Carrying TMPRSS3 Variants

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Background: Mutations in TMPRSS3 cause congenital or postlingual hearing loss (DFNB8/10), and defective Tmprss3 proteins are linked to hair-cell loss and altered endocochlear potential. Complex genotype–phenotype correlations in patients with TMPRSS3 variants, and poor cochlear-implant outcomes in some, have impeded our understanding of and treatment plan for TMPRSS3-associated recessive deafness. Since gene therapy has shown promise for treating hearing loss in aged mice with Tmprss3 mutations, we developed a human induced pluripotent stem cell (hiPSC)–derived inner-ear organoid model from TMPRSS3 patients to study the effects of patient-specific mutations on human hair cells.

Methods: hiPSC lines were reprogrammed from blood samples of patients carrying a) a homozygous c.208delC variant and b) compound heterozygous c.1333G > A and c.1273G > A variants; the former had congenital hearing loss, whereas the latter developed postlingual hearing loss. Inner-ear organoids were generated from mutant hiPSC lines and from their isogenic control lines.

Results: Organoid growth and hair-cell development were monitored by immunocytochemistry (ICC) using SOX2 and MYOSIN-7A antibodies. Hair-cell loss was assessed by ICC of cleaved caspase-3 to define the timepoint at which hair-cell degeneration occurs.

Conclusions: We established patient-derived inner ear organoids carrying TMPRSS3 variants for determining the timeline of hair cell degeneration, as well as the role of TMPRSS3 in the human inner ear. This model system will be critical for developing gene therapies for human inner ear disorders.

SU101. Single-Cell RNA Sequencing Reveals Hypoxia-Mediated Transcriptomic Alterations in Vestibular Schwannoma

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Background: Vestibular schwannomas (VS) are benign tumors of the vestibulocochlear nerve, most commonly driven by inherited or somatic mutations in the NF2 gene. Despite their benign histology, VS frequently cause debilitating symptoms, including progressive hearing loss and vestibular dysfunction. Like many solid tumors, VS exhibit regions of intra-tumoral hypoxia. Hypoxia triggers a transcriptional program mediated largely by hypoxia-inducible factor 1 α (HIF-1 α), which has been linked to tumor progression, treatment resistance, and poor outcomes across a variety of cancers. In VS, HIF-1 α expression has been detected, and its inhibition has been shown to suppress tumor growth, suggesting that hypoxia may play a critical role in tumor progression. However, while prior studies have implicated hypoxia in VS progression, the broader impact of hypoxia on the tumor microenvironment and transcriptomic profiles of VS has not been characterized. In this study we aim to investigate how hypoxia influences cellular heterogeneity and gene expression patterns in primary VS cells and identify pathways that may be related to tumor growth and survival.

Methods: Patient-derived VS tumor chunks were enzymatically dissociated and cultivated in proliferative media at room air (21% O₂) and hypoxia (1% O₂). Cells were imaged using light microscopy every week for ~1 month. Cell cultures were then dissociated into single-cell suspensions and subjected to single-cell RNA sequencing (scRNA-seq) using the 10x Genomics Chromium platform. Libraries were prepared using the Chromium Single Cell 3' reagent kit and sequenced on an Illumina platform. Raw data were processed using the Cell Ranger pipeline to generate gene-cell count matrices. Downstream analyses were performed in Seurat, including quality control, normalization, dimensionality reduction, clustering, and differential expression analyses. Immunocytochemistry (ICC) was performed for Schwann cell markers.

Results: Across both conditions, scRNA-seq captured transcriptional profiles from 14,770 high-quality single cells. We identified distinct cellular subpopulations, transcriptional profiles, and enriched pathways between 21% and 1% O₂. The expression of Schwann cell markers will be measured using ICC.

Conclusions: We performed single-cell transcriptomic characterization of primary VS cultivated in room air and in hypoxia. Results will demonstrate how hypoxia shapes cell heterogeneity and transcriptomic profiles in VS. Furthermore, findings in this study may identify candidate pathways for future therapeutic treatments for VS.

SU102. Auditory Temporal Responses of Mutant Mice and Diagnosis of Hearing Loss Pathology

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Background: As the search for treatments for hearing impairment gathers pace, it is becoming increasingly important to develop reliable tools to diagnose the underlying pathology so that the appropriate treatment method may be delivered. We use mutant mice to aid the development of non-invasive physiological tests that may be transferrable into a clinical setting. These mice carry different single gene mutations and have known sites of lesion covering the three major

categories of sensorineural hearing impairment described by Schuknecht and Gacek (1993); Sensory (hair cell dysfunction), Metabolic (stria vascularis dysfunction), and Neural (auditory neuron defects).

Methods: Amplitude Modulation Following Responses (AMFR) were evoked by stimuli comprised of 12kHz (or 24 kHz) carrier tones presented at 80dB SPL (100ms duration, 5ms rise/fall time), which were 100% sinusoidally amplitude-modulated at rates (modulation frequency, fm) from 100-1750Hz, and by presentation of 1000 Hz fm across stimulus levels from 0 – 95 dB SPL. We calculated AMFR magnitude, phase, group delay and synchrony coefficient as a function of fm or stimulus level (eg Shaheen et al. 2015). Auditory Brainstem Responses (ABRs) and thresholds were recorded in the same mice.

Results: AMFR amplitude vs fm transfer functions recorded at 80dB SPL were typically multi-peaked in normal hearing littermate control mice, having peaks with a range close to modulation rates of 300 Hz, 600 Hz and 1000 Hz. *Klhl18* < lowf > (Inner Hair Cell dysfunction), *Mir96* < *tm3.1Wtsi* > (Inner and Outer Hair Cell dysfunction), *Slc26a5* < *tm1-Cre* > (Prestin, OHC dysfunction), and *Wbp2* < *tm2a* > (dysfunctional innervation) mutant mice all showed pronounced reductions in AMFR amplitudes and synchrony at these three fm ranges. *Sgms1* < *tm1b* > and *S1pr2* < *stdf* > mutant mice (both with a stria vascularis dysfunction resulting in a reduced endocochlear potential) showed reduced AMFR amplitudes and synchrony across a more limited span of fm in the 300 Hz and 600 Hz ranges, but in contrast to the other mutants examined, these AMFRs were almost indistinguishable from littermate controls at the higher modulation rates tested. AMFR amplitudes recorded at the lower and mid-range modulation rates across all mutant mice were correlated with the auditory brainstem response thresholds of these mice. However, at the higher modulation rates, amplitudes recorded from strial mutant mice were more comparable to controls. Typically, across all pathologies, mice with higher ABR threshold generated lower amplitude AMFR, and those with lower ABR thresholds generated higher amplitude AMFRs.

Conclusions: Reduced AMFR amplitudes in hearing-impaired mice may be a general feature across “neural” and “sensory” pathologies, in contrast to the normal AMFR amplitudes seen at higher modulation rates in “metabolic” impairment. These observations may help in the development of a non-invasive physiological diagnostic test battery to differentiate different sites of pathology underlying inner ear sensorineural hearing loss and help to inform better treatment strategies for individuals.

SU103. A Simplified Human Full-Head Model for Investigating Electric-Acoustic Interaction With Intra- and Extra-Cochlear Electric and Acoustic Stimulation

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Background: Computational models of auditory nerve activity constitute indispensable tools for elucidating the neural mechanisms of sound perception. They enable systematic investigation of

the complex interactions between electric and acoustic stimulation (EAS) within the auditory system. A computational model has been substantially extended to represent more accurately the anatomical and electrical properties of the full human head. The extended model incorporates detailed 3D representations of the outer, middle, and inner ear, as well as the brain, forming a population-based full-head model. The model can be used to simulate intra- and extra-cochlear electric stimulation of the auditory system as well as the interactions between electric and acoustic stimulation. The goal of the computation model is to optimize the amount of current flow inside the cochlea by varying the location of the stimulating and reference electrode and assess interactions between electric and acoustic stimulation.

Methods: Finite element method (FEM) simulations were conducted under monopolar stimulation modes for both intra- and extra-cochlear electrodes. A novel computational framework was implemented to simulate both acoustically evoked compound action potentials (aCAPs), electrically evoked compound action potentials (eCAPs) and Electrocochleography (ECoChG) in humans, with a specific focus on interactions arising from combined electric-acoustic stimulation (EAS) at the level of the auditory nerve. Key variables simulated include: transimpedance matrices (TIMs) of intracochlear electrodes, intra- and extra-cochlear voltages and currents, auditory nerve activity, and the resulting aCAPs, eCAPs and ECoChG.

Results: The model reliably reproduced transimpedance matrices (TIMs) and response trends consistent with those reported in cochlear implant users with the same electrode array. Novel intra- and extra-cochlear current pathways through the scala tympani were identified, revealing distinct voltage and current density distributions between the two stimulation modes. The framework is capable of simulating electrocochleography (ECoChG) responses resembling human recordings and providing a complementary means to assess electric-acoustic interactions. Preliminary simulations confirmed measurable interaction effects at hair cell and auditory nerve level. Simulated compound action potential (CAP) waveforms from intracochlear electrodes closely matched those observed in human recordings. A marked reduction in eCAP responses was observed under both intra- and extra-cochlear stimulation when an acoustic masker was applied. Mechanisms underlying EAS interactions in CAP responses were systematically investigated, with spike interactions in the apical cochlea and ectopic stimulation of the auditory nerve trunk emerging as the most plausible explanations.

Conclusions: This simplified full-head human model represents a promising tool for investigating electric-acoustic interactions and optimizing electric stimulation for both intra- and extra-cochlear electric stimulation in cochlear implant users. These results are relevant to create a diagnostic to diagnose hearing loss and a prosthetic device that augments acoustic hearing with extra-cochlear electric stimulation.

SU104. ADARB1 as a Nuclear Marker of Spiral Ganglion Neurons Across Species and Age

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Background: Spiral ganglion neurons (SGNs) are the primary neurons responsible for transmitting auditory signals from the inner ear to the brain. Degeneration of SGNs occurs in various conditions including auditory neuropathies, traumatic brain injury, and aging, thus

contributing to acquired hearing loss. Precise characterization and quantification of SGNs are critical for advancing the understanding of auditory pathology and evaluating potential therapeutic interventions. Here we show that immunolabeling for ADARB1, an RNA editing enzyme enriched in the nuclei of glutamate receptor-expressing neurons, is a reliable method for SGN identification and quantification in young adult mice. We compared manual SGN counts obtained via neurofilament heavy chain (NFH) immunolabeling to automated counts generated with ADARB1 immunofluorescence. We further characterized ADARB1 immunoreactivity in aged mice (24 months), as well as in human and macaque inner ear tissues. Co-labeling sections for ADARB1 and HuD, NFH, GATA3, or SOX2 was undertaken to confirm neuronal identity.

Methods: Mid-modiolar cochlear sections were obtained from 3-week-old CD1 mice, 24-month-old C57Bl/6 mice, and archived (> 5 years) temporal bone sections from macaques (4 years-old) and human cadavers of varying ages (all > 40 years of age). The human, macaque, and 24-month-old mouse samples were immunolabeled with antibodies against ADARB1 and neurofilament heavy chain (NFH), while the 3-week-old mouse samples were immunolabeled with antibodies against ADARB1, NFH or HuD, and either SOX2 or GATA3. Micrograph images were acquired using laser scanning confocal microscopy with a 40X objective. Image processing, including manual thresholding and noise reduction, was performed using ImageJ (Fiji).

Results: Within Rosenthal's canal, ADARB1 immunolabeling yielded specific and robust staining of spiral ganglion neurons (SGNs), allowing for reliable automated quantification using the analyze particles function in ImageJ. Comparison across over 30 sets of images yielded a Lin's concordance correlation coefficient > 0.95, indicating strong agreement between manual and automated counts. Strong ADARB1 expression was observed in surviving SGNs in 24-month-old mouse samples demonstrating utility in a context where hair cell and SGN loss were evident. ADARB1 labeling of SGN nuclei was also prominent in macaque and human sections, though some variability was noted in the cadaveric samples. Colocalization analyses demonstrated that ADARB1 labels both type I and type II SGNs, as evidenced by overlap with HuD, NFH, and GATA3-labeling. Conversely, a lack of colocalization between ADARB1 and SOX2 indicated minimal ADARB1 expression in glial cells.

Conclusions: These studies demonstrate the specificity and robust expression of ADARB1 in spiral ganglion neurons (SGNs) and validate the effectiveness of ADARB1 labeling for automated quantification of SGNs, indiscriminate of type, and across diverse mammalian species.

SU105. Reimbursement for Laryngology Procedures Performed in Facility-Based Versus Non-Facility-Based Settings: Trends and Implications for Practice

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Background: The Centers for Medicare and Medicaid Services (CMS) create a method of payment for enrolled healthcare providers called the Medicare Physician Fee Schedule (MPFS). Procedures have separate reimbursement rates for physician services provided in facility and non-facility-based settings. When procedures are performed in facility-based settings, CMS

reimburses a technical fee and allows devices to be billed for separately from the procedure (e.g., laser fibers and balloons.) Conversely, devices used in non-facility-based settings cannot be billed separately from the procedure CPT code; thus, when assessing profitability, the cost of these is subtracted from the total reimbursement. Hence, device cost can be prohibitive in performing these procedures in the non-facility-based setting, forcing procedures to be done in higher-cost locations such as the operating room (OR) or surgery center. There are limited studies examining reimbursement patterns in these two different practice settings. Thus, this study compares the reimbursement rates of commonly performed office-based Laryngology procedures, considering the cost of devices, fillers, and pharmaceuticals, for facility-based versus non-facility-based settings.

Methods: The MPFS Look-Up Tool was used to determine the reimbursement for 11 common office-based Laryngology clinic procedures for facility-based and non-facility-based locations. The technical fee for the facility-based setting was provided by a CMS billing consultant. The cost of pharmaceuticals, devices, or fillers was subtracted from the reimbursement for each procedure to determine the profit margin per procedure, which was calculated for all procedures.

Results: Procedures requiring balloon devices, injection augmentation materials, and laser fibers in the non-facility setting reimbursed significantly lower (76.5%, 62.6 – 95.9%, and 99.7% lower, respectively) compared to facility settings that receive both technical and professional fee reimbursements ($p < 0.05$). When accounting for procedural materials (e.g., injectable filler), profit margins were considerably lower in the non-facility setting (40.5%, 4.8 – 60.6%, and 2.3% relative profit margins, respectively.) All other procedures were profitable in both settings but more so in the facility setting (52.5 – 87.3% reductions in reimbursement in non-facility versus facility settings.)

Conclusions: Overall healthcare utilization cost of Laryngology procedures in a clinic setting is favorable to an OR setting, but clinicians who practice in non-facility-based clinics are at risk for a monetary loss due to lower reimbursements and expensive devices. Performing office-based procedures is an important component in providing equitable and timely treatment. If reimbursement rates continue to bias in favor of facility-based settings, there is a risk of non-facility-based clinics no longer performing procedures or of increasing overall healthcare costs by shifting them to the OR setting.

SU106. Effect of IL-8 Inhibition on Cancer Cell Proliferation and Migration in Oral Cavity Squamous Cell Carcinoma

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Background: Oral cavity squamous cell carcinoma (OCSCC) is the most common SCC of the head and neck and the 6th most common cancer worldwide. A significant poor prognostic factor in OCSCC is perineural invasion (PNI), which occurs in an estimated 24-31% of cases and can lead to taste alterations. PNI is the spread of cancer into or around nearby nerves, and when present, is associated with higher locoregional recurrence and worse disease-free and overall survival. The mechanisms underlying PNI is still unresolved, however recent evidence suggest

that both Schwann cells and cancer secrete IL-8, which may have a role in promoting OCSCC cell migration and proliferation. We hypothesize inhibiting IL-8 signaling in OCSCC cells will reduce cancer cell proliferation and migration.

Methods: Human OCSCC (SCC9, UCSFOT1109) and Schwann cell lines (HS11, FHSC) were cultured in maintenance media. Cell-conditioned supernatants were collected at 48 hours, and antibody arrays were utilized to profile the secretion of 80 cytokines. A top secreting cytokine was determined to be interleukin-8 (IL-8). Subsequently, OCSCC cells were cultured in serum-reduced conditions and exposed to 0 or 1000 ng/ml anti-IL-8 neutralizing antibody for 12, 24, and 48 hours. Transwell and scratch assays were performed to measure the effect of anti-IL-8 on chemotaxis and cell migration. Viability assays were utilized to determine the impact of anti-IL-8 on cell proliferation.

Results: IL-8 was highly secreted by SCC9, UCSFOT1109, HS11, and FHSC. Anti-IL-8 antibody significantly reduced gap closure on scratch assay in both OCSCC cell lines at 4 hours; however, the effect did not persist at longer time points. Additionally, on transwell assay, anti-IL-8 antibodies also significantly reduced the number of migrated cells at all time points in SCC9 and early point of 12 hours in UCSFOT1109. Anti-IL-8 antibody did not significantly affect the proliferation of either OCSCC cell lines at any time point.

Conclusions: We found that OCSCC and Schwann cells secrete high levels of IL-8 into the tumor-nerve microenvironment. Inhibiting IL-8 signaling in vitro using anti-IL-8 neutralizing antibodies reduced migration, but did not affect proliferation, of OCSCC. Further investigation into the role of IL-8 in the tumor-nerve microenvironment may provide additional insights into cancer spread and perineural invasion and may reveal novel therapeutic targets for treatment of OCSCC.

SU107. Hypoxia-Induced BNIP3 and HIF-1 α Reveal Metabolic Vulnerabilities in Vestibular Schwannoma

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Background: Under hypoxic conditions, cancer cells can undergo metabolic reprogramming through the induction of hypoxia-inducible factors (HIF-1 α) and Bcl-2/adenovirus E1B 19 kDa interacting protein 3 (BNIP3), enabling them to generate energy and biosynthetic precursors needed for survival. Vestibular schwannomas (VS), benign tumors of the vestibulocochlear nerve, are known to express HIF-1 α , which promotes angiogenesis and tumor growth. However, the metabolic response of VS to hypoxia, and how it compares to wild-type Schwann cells (WT-SCs) and merlin-deficient Schwann cells (MD-SCs), remains poorly understood. This study aims to define how oxygen tension influences metabolism and hypoxia-related signaling across these cell types.

Methods: Primary VS cells were derived from patient tumors, while MD-SCs were generated by introducing NF2 mutations into WT-SCs derived from fetal tissue and human induced

pluripotent stem cells. VS, WT-SCs, and MD-SCs were cultured for 72 hours under normoxia (21% O₂), physioxia (5% O₂), or hypoxia (1% O₂). Metabolic activity was assessed using CellTiter-Glo assays, and immunocytochemistry (ICC) was performed for BNIP3 and HIF-1 α . Data were analyzed with descriptive statistics and one-way ANOVA.

Results: VS cells demonstrated the lowest ATP production at 1% O₂, consistent with reduced oxidative phosphorylation under hypoxia. A majority of VS cultures exhibited robust upregulation of HIF-1 α and BNIP3 at 1% O₂, with concordant expression patterns within samples. In contrast, WT-SCs and MD-SCs did not show significant hypoxia-induced upregulation of these markers.

Conclusions: Most primary VS cells upregulate BNIP3 and HIF-1 α under hypoxia, demonstrating their ability to undergo metabolic reprogramming and adapt to oxygen-limited conditions. In contrast, WT-SCs and MD-SCs did not exhibit this response, suggesting that NF2 loss alone is insufficient to drive these changes. These results highlight hypoxia-driven adaptations as a defining feature of VS biology and point to metabolic pathways as promising therapeutic targets for VS.

SU108. Global Mapping of Clinical Trials in Hearing Disorders (2019–2024): A Systematic Mapping Review

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Background: Hearing disorders pose a significant public health burden, yet no comprehensive mapping of clinical research exists. This project systematically catalogues and analyzes clinical trials over the past five years to identify evidence gaps and redundancies in prevention and treatment strategies in the field.

Methods: We conducted a systematic search of four international trial registries, the US ClinicalTrials.gov registry, the Australian New Zealand Clinical Trials Registry (ANZCTR), the ISRCTN registry in the United Kingdom and the European EU clinical Trials Register and Clinical Trials information system (EUCTR/CTIS), identifying interventional studies first posted between January 1, 2019, to August 14, 2024. Eligible studies evaluated interventions for hearing disorders across all ages and severities. Data were extracted using a standardized PICO (Population, Intervention, Comparison, Outcome) framework, covering study design, populations, interventions, outcomes, sample size, funding, and geographical distribution. Additional study characteristics, including randomization, trial phase, recruitment status, and number of arms, were recorded, along with the results posting and associated publications of completed studies. Two epidemiologists independently screened records (30% duplicate screening for quality assurance). A hierarchical classification system was developed for interventions (pharmacological, devices, gene therapy, etc.) and hearing conditions by laterality,

onset, and severity following WHO criteria and the International Bureau for Audiophonology BIAP Recommendation 02/1).

Results: Of 1,679 identified records, 547 trials met inclusion criteria (67% excluded, primarily for non-interventional designs or off-topic focus). Most were randomized (58.9%), assessed therapeutic interventions (66%), and involved adults (81.6%). Common conditions included sensorineural hearing loss (19.9%), tinnitus (19.2%), and presbycusis (4.3%). Device-based interventions were the most prevalent (37.1%), followed by non-pharmacological therapies (17.1%) and pharmacological treatment (10.3%).

Data on hearing loss characteristics were variably reported. Regarding the type of hearing loss, 9.1% were unilateral, 19.2% bilateral, 11.6% included both types, and 1.6% had normal hearing; 58.5% were not reported.

Only 13.3% of completed trials (n=210) had results posted in registries; 31 were linked to PubMed publications. Geographically, the U.S. (34.7%), France (6.8%), and Australia (6.8%) led research output, with 85.7% of studies being nationally focused. Descriptive statistics and an interactive evidence map were generated.

Conclusions: This first systematic map of hearing disorders' trials reveals disparities in research focus, with underrepresentation of pediatric populations and low publication rates despite trial completion. The interactive dashboard highlights regional and thematic gaps. Such mapping is essential for identifying areas of research that have already been thoroughly explored, thereby limiting duplication, as well as highlighting persistent gaps in the scientific literature. This open-access platform will serve as a data-driven decision-making tool, facilitating the identification of research priorities and optimizing the impact of scientific work in the field of hearing health.

SU109. Incidence and Risk Factors of Postoperative Sensorineural Hearing Loss After Cholesteatoma Removal

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Background: While cholesteatoma and chronic ear disease are primarily associated with conductive hearing loss, all otologic surgical intervention carries a risk of sensorineural hearing loss (SNHL). This study aims to evaluate the incidence of SNHL following cholesteatoma surgery and identify potential risk factors contributing to its development.

Methods: A retrospective chart review was conducted of 345 patients who underwent surgery for cholesteatoma removal between January 2021 and June 2024. Data included patient demographics, prior ossicular reconstruction, prior surgery, extent of surgery, disease characteristics, and audiometric data. Multivariable logistic regression was utilized to assess associations between potential risk factors and the development of SNHL. SNHL was defined as a ≥ 20 dB drop in bone conduction threshold at any frequency from 250-4000Hz on postoperative audiograms ranging from 2-24 months.

Results: 345 patients [mean age 51.2 ± 17.3 years; 187 (54.2%) males] were analyzed. 301 had preoperative mixed or conductive hearing loss, mean air-bone gap (ABG) was 26.1 dB, and 182

(61.7%) demonstrated postoperative improvement in the ABG (mean 21.3 dB). 59 patients (17.1%) developed postoperative SNHL, with 56 patients (16.2%) demonstrating SNHL within the first year. 3 patients (0.9%) presented with SNHL at 12-24 months. Prior ossicular prostheses (OR 3.74 [1.08, 13.2]; $p=0.037$) was associated with increased risk of postoperative SNHL, whereas new prosthesis placement (OR 0.19 [0.055, 0.545]; $p=0.0041$) and a canal wall down approach (OR 0.268 [0.110, 0.614]; $p=0.0026$) was associated with a decreased likelihood when controlling for race and surgeon. Revision surgery, age, sex, and race did not reach significance. The 17.1% incidence of SNHL, primarily in the first year, constitutes a clinically meaningful risk profile that should inform prognostic counseling and follow-up planning.

Conclusions: These findings provide contemporary incidence and risk profiling relevant to otologic surgical planning, prognosis, and patient expectation setting. SNHL is a non-negligible complication after cholesteatoma surgery, with patients' risk profiles influenced by a prior and current prosthesis placement as well as the extent of surgical intervention. Key takeaways include: (a) postoperative SNHL > 20 dB at any frequency after cholesteatoma surgery is not rare (~1 in 6); (b) most cases appear within 12 months; (c) prior ossicular prosthesis is a salient risk marker; and (d) in this cohort, canal wall down approach and new prosthesis placement correlate with lower odds of developing postoperative SNHL. While canal wall down approach generally entails a more extensive surgery, this association may reflect technique-mediated protection (e.g., less ossicular manipulation) or be explained by greater rates of preoperative SNHL (i.e., confounding by indication). Future research with larger patient cohorts may help optimize risk stratification, identify causal pathways, and develop hearing preservation protocols to enhance patient counseling and surgical management.

SU110. The Role of Concomitant Medications in Improving SPI-1005 Study Outcomes in Meniere's Disease Patients in a Phase 3 Randomized Placebo-Controlled Trial (STOPMD-3)

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Background: Meniere's disease (MD) involves episodic vertigo or dizziness, fluctuating low frequency hearing loss, and intermittent or constant tinnitus. No FDA-approved drug therapies exist for MD or its auditory or vestibular symptoms. Medical management with diuretics, betahistidine, and corticosteroids have demonstrated low evidence of efficacy. SPI-1005, a novel anti-inflammatory drug, has shown safety and efficacy in three MD studies: Phase 1b (N=39), Phase 2b (N=126), Phase 3 (N=221) randomized clinical trials (RCTs) after 21 or 28-days of oral treatment. In the Phase 3 RCT, SPI-1005 treatment showed significant improvements in two pre-specified primary efficacy endpoints involving low-frequency hearing and speech discrimination over placebo. Here, we present post-hoc analyses of the Phase 3 RCT involving common concomitant medications (CM) used to manage MD.

Methods: Adults (18-75 years) were enrolled at 11 US sites (NCT04677972) and were randomized to either SPI-1005 400 mg or placebo BID, for 28 days. Participants were allowed to continue existing CM, excluding corticosteroids. Pure-tone audiometry (PTA), words-in-noise (WIN), tinnitus-functional-index (TFI) including VAS-loudness (TL), vertigo-symptoms-scale (VSS), dizziness-handicap-inventory (DHI), and VAS-aural fullness (AFS) were assessed at

baseline, 28, 56, and 84-days. Compliant participants entered OLE and received SPI-1005 400 mg BID, for up to 12-months, and were reassessed every 3-months. Improvements in low-frequency PTA (LFPTA) and WIN scores in either ear from baseline were applied: ≥ 10 dB at 250, 500, or 1000 Hz; and ≥ 4 -words in WIN at 84-days. CMs taken by $> 15\%$ of participants were identified and stratified.

Results: 191 Per-Protocol subjects (mean age 55.5; 103 males/88 females) were analyzed. CM use was 94 (49.2%) for hydrochlorothiazide, 74 (38.7%) for triamterene, 44 (23.0%) for hydrochlorothiazide/triamterene, 38 (19.9%) for diazepam, 35 (18.3%) for betahistine, and 34 (17.8%) for meclizine.

LFPTA improvements for SPI-1005 vs placebo with hydrochlorothiazide (27/48, 56.3%, vs 15/46, 32.6%, $p=0.0243$); triamterene (22/36, 61.1%, vs 12/38, 31.6%, $p=0.0190$); hydrochlorothiazide/triamterene (12/17, 70.6%, vs 8/27, 29.6%, $p=0.0128$); diazepam (12/16, 75.0%, vs 9/22, 40.9%, $p=0.0516$); betahistine (9/18, 50.0%, vs 7/17, 41.2%, $p=0.7380$); meclizine (11/18, 61.1%, vs 5/16, 31.3%, $p=0.1005$).

WIN improvements for SPI-1005 vs placebo with hydrochlorothiazide (21/48, 43.75%, vs 15/46, 32.6%, $p=0.2950$); triamterene (16/36, 44.4%, vs 13/38, 34.2%, $p=0.4757$); hydrochlorothiazide/triamterene (9/17, 52.9%, vs 10/27, 37.0%, $p=0.3588$); diazepam (4/16, 25.0%, vs 7/22, 31.8%, $p=0.7292$); betahistine (8/18, 44.4%, vs 3/17, 17.6%, $p=0.1464$); meclizine (9/18, 50.0%, vs 4/16, 25.0%, $p=0.1717$).

During OLE, subjects treated with SPI-1005 and CM continued to show LFPTA (27-65%) and WIN (18-50%) improvements at 3- to 12-months. No significant differences were found between SPI-1005+CM vs SPI-1005 alone.

Conclusions: Adjunctive therapy with SPI-1005 on to the existing medical management for MD involving diuretics (hydrochlorothiazide, triamterene, or hydrochlorothiazide/triamterene) demonstrated significant improvements in low-frequency hearing but not speech discrimination over placebo. Non-significant improvements were observed for diazepam, betahistine, and meclizine.

SU111. Systemic Ebselen Treatment Prevents Amikacin Induced Ototoxicity and Nephrotoxicity in Mice

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Background: Aminoglycosides (AGs), such as tobramycin and amikacin, commonly used to treat recurrent pulmonary infections, are ototoxic and nephrotoxic. Our previous mouse studies showed that 14-days of amikacin (500 mg/kg/day) induced temporary threshold shifts (1-11 dB), tinnitus (24%), and hyperacusis (82%) which were attenuated by ebselen (20 mg/kg/day). The present study determined whether 28-days of amikacin increases the severity/duration of ototoxicity and if ebselen prevents amikacin-induced ototoxicity and nephrotoxicity.

Methods: Adult CBA/CaJ mice (N=32) 3-7 months old were divided into three groups: 1) Control (N=10); 2) Amikacin (N=10); and 3) Amikacin+Ebselen (N=12). Groups 2 and 3 received vehicle or ebselen (20 mg/kg/day x 28-days i.p.) followed by amikacin (500 mg/kg/day

x 28-days s.c.) 30-minutes later, respectively. Auditory brain-stem response (ABR) and acoustic startle response (ASR) were performed at baseline and at 4-, 8-, 12-, and 16- weeks from the start of treatment. ABR was also performed at 35-weeks. Clinically relevant change (CRC) using ABR threshold shift criteria (hearing loss), gap-induced-prepulse-inhibition of the acoustic startle changes (tinnitus), and startle magnitude shifts (hyperacusis) were analyzed. Cochlear histopathology was performed using Myosin-7a immunolabeling to identify outer hair cell (OHC) loss. Serum samples were collected at baseline and 4-weeks for blood urea nitrogen (BUN) and creatinine (Cr) levels (nephrotoxicity).

Results: Control mice showed permanent threshold shifts (PTS) of 1–12 dB at multiple frequencies and time-points. CRC rates were 25%, 20%, 15%, 35%, and 65% at 4-, 8-, 12-, 16-, and 35-weeks, respectively. Tinnitus incidence was 0%, 0%, 0%, and 10% at 4-, 8-, 12-, and 16-weeks, respectively with no observed hyperacusis.

28-day amikacin resulted in PTS of 14–25 dB at multiple low frequencies and time-points. CRC rates were 75%, 60%, 45%, 50%, and 94%, respectively. Tinnitus incidence was 22%, 22%, 33%, and 0%, respectively with no hyperacusis.

28-days ebselen significantly reduced amikacin-induced PTS to 4–20 dB at multiple low frequencies and time-points: 4-weeks ($p=0.0029$) and 8-weeks ($p=0.0032$). CRC rates were also reduced to 29% ($p=0.0056$), 8% ($p=0.0003$), 33%, 46%, and 79%, respectively. Tinnitus incidence was 13%, 25%, 0%, and 13%, respectively with no hypoacusis.

Ebselen prevented amikacin-induced changes in Cr levels at 4-weeks, -0.042 vs $+0.030$ mg/dL ($p=0.0216$). BUN levels did not significantly change from baseline levels in either group.

Conclusions: 28-days of amikacin increased the severity and duration of ototoxicity that was significantly reduced by ebselen. Amikacin-induced nephrotoxicity (increased Cr) was significantly reduced by ebselen. Interestingly, no hyperacusis was observed in any group and may be a consequence of greater PTS. These findings are consistent with recent clinical data from a Phase2-RCT involving adults receiving 14-days of IV-tobramycin. These results extend our earlier findings involving 14-days of amikacin using a dose schedule that mimics the clinical treatment of multi-drug resistant TB and NTM.

SU112. Furosemide Attenuates Noise-Induced Hearing Loss and Potentiates Preventive Effect of N-Acetylcysteine

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Background: Noise-induced hearing loss (NIHL) is a major cause of sensory hair cell damage, primarily driven by dysregulation of reactive oxygen species (ROS) homeostasis. Antioxidants such as N-acetylcysteine (NAC) have demonstrated protective effects, underscoring the role of ROS in NIHL. However, no FDA-approved pharmaceutical treatment currently exists, partly due to the complexity of cochlear damage mechanisms and the restrictive nature of the blood-labyrinth barrier (BLB), which limits drug delivery to the inner ear. This study investigated the protective mechanisms of furosemide (FRS) against NIHL and its potential to enhance the efficacy of NAC.

Methods: FVB/NJ mice were pretreated with a single dose of FRS (200 mg/kg) prior to noise exposure. Auditory brainstem response (ABR) thresholds and endocochlear potential (EP) were measured to assess auditory function. The permeability of the stria vascularis (SV) to small molecules and protein-bound tracers was evaluated. The combined effect of FRS and NAC on outer hair cell (OHC) survival and hearing prevention was also examined.

Results: FRS pretreatment temporarily reduced EP without elevating ABR thresholds and significantly attenuated NIHL. The most effective protection was observed when FRS was administered immediately before noise exposure. FRS selectively increased SV permeability to small molecules, facilitating drug delivery without affecting larger protein-bound tracers. Combined FRS and NAC treatment enhanced antioxidant effects, resulting in additive protection against OHC loss and NIHL, with near-complete preservation of OHCs.

Conclusions: Furosemide effectively attenuates NIHL by transiently modulating cochlear physiology and enhancing drug permeability across the BLB. Its combination with NAC offers a promising strategy for preventing NIHL, highlighting a potential therapeutic approach for clinical application.

SU113. Insulin-Like Growth Factor 1 Protects Cochlear Outer Hair Cells Against Cisplatin by Reducing Oxidative Stress and Inhibiting Apoptosis

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Background: Cisplatin is a widely used and highly effective anti-neoplastic agent included in numerous oncology treatment protocols for both pediatric and adult patients. However, one of its most serious adverse effects is ototoxicity, which currently lacks any clinically approved preventive or therapeutic interventions. Histological studies have shown that cochlear lesions following cisplatin administration are particularly prominent in outer hair cells (OHCs) located in the organ of Corti, contributing significantly to irreversible sensorineural hearing loss and decreased quality of life. Our previous studies demonstrated that insulin-like growth factor 1 (IGF1) has a protective role in the cochlea, promoting the survival of auditory hair cells (HCs) after diverse forms of cochlear injury, including noise trauma, ischemia, surgical insult, and exposure to aminoglycoside antibiotics. These findings prompted us to explore whether IGF1 can protect cochlear sensory HCs against cisplatin-induced toxicity. In this study, we utilized a cochlear explant culture model derived from postnatal day 2 mice to evaluate the otoprotective efficacy of IGF1 against cisplatin exposure.

Methods: To determine the protective effects of IGF1 against cisplatin, the explants grown in the medium containing 10 μ M cisplatin were treated for 36 h with 1 or 5 μ g/ml recombinant human IGF1. To assess whether the protective effects were induced by IGF1 binding to its receptor (IGF1R), the receptor-competitive antagonist or the IGF1R tyrosine kinase inhibitor was used. Apoptosis in the cochlear epithelium was determined by treating the explants with 10 μ M cisplatin with or without 5 μ g/ml IGF1 and immunostaining with anti-cleaved caspase 3 antibodies. The effects on the cell cycle were examined by adding 3 μ g/ml 5-bromo-2'-deoxyuridine (BrdU) to the explants treated with 10 μ M cisplatin with or without 5 μ g/ml IGF1

for 36 h. To determine the antioxidant capacity of IGF1, the glutathione to glutathione disulfide (GSH/GSSG) ratios of the cochlear supernatant were measured using the GSH/GSSG-Glo assay.

Results: Administration of IGF1 to the explants grown in the medium containing cisplatin markedly protected outer HCs from cisplatin-induced damage. Pharmacological inhibition of IGF1R using an IGF1R antagonist or inhibitor markedly attenuated the protective activity of IGF1, indicating that IGF1R is specifically required for IGF1 effects in HCs against cisplatin. As a protective mechanism against cisplatin, we found that the administration of IGF1 reduces cisplatin-induced oxidative stress. IGF1 effects on the maintenance of HC numbers are achieved by inhibiting the apoptosis of HCs, not by inducing the proliferation of HCs or supporting cells (SCs).

Conclusions: These data suggest that IGF1 may serve as a promising and clinically applicable therapeutic strategy for preventing cisplatin-induced ototoxic damage in patients undergoing chemotherapy.

SU114. Predicting the Ototoxic Risk of Fda-Approved Drugs Through Machine Learning and in Vivo Approaches

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Background: The ototoxic potential of drugs is rarely evaluated during pre-clinical development or clinical trials, resulting in hearing loss as an underrecognized adverse effect of many medications in use. Ototoxicity likely remains underreported for numerous FDA-approved drugs due to a lack of predictive tools or mandatory regulation. Our goal is to predict the ototoxic potential of FDA-approved drugs using a machine learning (ML) model and to validate the predictions experimentally in the zebrafish lateral line.

Methods: The ML model was trained on a curated dataset of 283 compounds with established ototoxic or non-ototoxic classifications derived from published literature. This model was based on an F1-optimal threshold, high-recall screening, and well-calibrated probabilities, with flexible risk-tolerance tuning for new compound libraries. We applied the model to a DrugBank database of 2423 FDA-approved drugs, then validated a subset of model predictions in larval Brn3c:mGFP transgenic fish, which express hair cell-specific GFP. The larvae were exposed to various concentrations of predicted ototoxins and compared to vehicle-treated controls, followed by hair cell quantification using fluorescence microscopy.

Results: Our model predicted that among the FDA-approved drugs, 692 compounds have ototoxic potential based on their physicochemical and pharmacological properties. Of the top 60 compounds, 18 were known ototoxins such as aminoglycosides and loop diuretics. From the predicted list, we selected 30 drugs with a model-assigned probability of ototoxicity between 67–100% for experimental validation. Known ototoxins were excluded to focus on compounds previously not associated with auditory toxicity. To date, we have experimentally tested eight compounds and confirmed that two drugs, Dantrolene and Nitrofurantoin, were ototoxic. A third, Fruquintinib, showed hair cell loss only at the highest dose, suggesting general cytotoxicity. Among the 32 evaluated drugs, the model correctly predicted 20 as ototoxic (18 known and 2 experimentally validated), corresponding to 62% alignment with expected outcomes.

Conclusions: Preliminary findings demonstrate that ML-based predictions for unrecognized ototoxic compounds need improvement, as 2 out of 8 tested drugs were confirmed as ototoxins in zebrafish. Further experiments will explore whether the confirmed ototoxic drugs from the predicted list enter via mechanotransduction (MET) channels, affect ribbon synapses, and ultimately impact hair cell function. Our future work will focus on refining the model to achieve > 90% prediction confidence and developing it into an accessible, robust screening tool for drug-induced ototoxicity.

SU115. Prevention of Hearing Loss in the Cochlea of Type 2 Diabetes Model Mice

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Background: The number of patients with diabetes mellitus is increasing worldwide due to changes in lifestyle and social environment. Diabetic patients are more likely to develop hearing loss (diabetes-related hearing loss), which often occurs from a relatively young age and significantly reduces quality of life (QOL). To clarify the mechanisms and effective preventive strategies of diabetic hearing loss, we investigated auditory function, cochlear histopathology, and preventive interventions using a type 2 diabetes mouse model.

Methods: TSOD mice, a type 2 diabetes model, and control mice were used. Auditory function was evaluated periodically by auditory brainstem response (ABR). After evaluation, cochleae were harvested from the temporal bone, and vascular structures were examined by vascular staining along with assessment of hair cells and spiral ganglion cells. Preventive interventions included caloric restriction, administration of traditional Japanese herbal medicines (Bofutsusho-san and Dai-saiko-to), metformin, and SGLT2 inhibitors.

Results: TSOD mice exhibited hyperglycemia, obesity, and earlier onset of hearing loss compared with controls. In the cochlea, reduced capillary area was observed in the stria vascularis. Preventive interventions showed that caloric restriction and herbal medicine administration were effective, whereas metformin and SGLT2 inhibitors did not demonstrate significant protective effects compared with controls.

Conclusions: The number of patients with diabetes mellitus is increasing worldwide due to changes in lifestyle and social environment. Diabetic patients are more likely to develop hearing loss (diabetes-related hearing loss), which often occurs from a relatively young age and significantly reduces quality of life (QOL). To clarify the mechanisms and effective preventive strategies of diabetic hearing loss, we investigated auditory function, cochlear histopathology, and preventive interventions using a type 2 diabetes mouse model.

SU116. Effects of Cholesterol Modulation on Cisplatin-Induced Hearing Loss

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Background: Cisplatin is a widely used and effective anticancer drug. However, it causes permanent sensorineural hearing loss in over 50% of treated patients. There are no FDA-approved therapies to prevent cisplatin-induced hearing loss (CIHL) in adults, highlighting a critical unmet clinical need. Previous studies suggest that statins, commonly prescribed cholesterol-lowering drugs, are associated with reduced incidence and severity of cisplatin-induced hearing loss. The mechanisms underlying the protective effect of statins are unclear; however, the hallmark activity of statins is inhibition of HMG Co-A reductase, resulting in reduced serum cholesterol. Thus, to begin to address the mechanisms underlying statin-induced protection, we asked whether a genetic model with reduced serum cholesterol is also protective.

Methods: Here we examine whether reduced plasma cholesterol confers protection against CIHL independent of statin treatment. We utilized mice lacking serine protease proprotein convertase subtilisin/kexin type 9 (Pcsk9 knockout mice) as a genetic model of reduced plasma cholesterol.

Results: We find that Pcsk9 KO mice have reduced cholesterol compared to their WT littermates. Pcsk9 KO mice were significantly protected against cisplatin-induced hearing loss, as reflected by significantly lower ABR and DPOAE threshold shifts relative to WT. Histological analyses confirmed preservation of cochlear outer hair cells (OHCs) in Pcsk9 KO mice treated with cisplatin, whereas WT mice showed significant OHC loss in the high-frequency cochlear regions. Finally, hearing loss positively correlated with baseline plasma cholesterol levels.

Conclusions: Together our data demonstrate that systemic cholesterol reduction provides significant protection against CIHL, and they suggest that the protective effect of statins against CIHL is, in part, mediated by cholesterol reduction.

SU117. Connexin30-Deficient Mice Increase Susceptibility to Noise via Redox and Lactate Imbalances

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Background: Noise significantly contributes to one-third of the global burden of hearing loss. The intricate interplay of genetic and environmental factors impacts various molecular and cellular processes that lead to noise-induced hearing loss (NIHL). Defective connexin 26 (Cx26) and connexin 30 (Cx30), encoded by Gjb2/Cx26 and Gjb6/Cx30, respectively, are prevalent causes of hereditary deafness. However, the role of Cx30 in the pathogenesis of NIHL remains unclear.

Methods: Cx30-deficient mouse models related to NIHL were established and subjected to audiological follow-up using auditory brainstem response (ABR) measurements. Cochlear morphology was analyzed via immunofluorescence, transmission electron microscopy, and toluidine blue staining. Changes in oxidative stress were observed and compared using dihydroethidium (DHE), 4-hydroxynonenal (4HNE), and western blot assays. Cochlear glucose transport and energy metabolism were assessed via 2-NBDG glucose uptake and ATP release assays. Metabolomic analysis was employed to investigate mechanisms underlying cochlear energy alterations.

Results: We observed that homozygous Cx30 knockout (Cx30 KO) mice exhibited poorer hearing recovery after noise exposure (97 dB mean sound pressure level for 2 h) and increased susceptibility to noise. In addition to the exacerbation of noise-induced damage to hair cells and synapses, Cx30 KO mice exposed to noise exhibited increased oxidative stress. The 2-NBDG assay showed a reduction in glucose levels associated with a decrease in gap junctions as well as a reduction in adenosine triphosphate release. Glucose metabolomics analysis further revealed that Cx30 KO mice had elevated lactate and NAD⁺ levels after noise exposure, thus worsening anaerobic oxidation from glycolysis.

Conclusions: Our study emphasizes that Cx30-deficient mice increase susceptibility to noise via redox and lactate imbalances in the cochlea.

SU118. The Cisplatin-Induced Hearing Loss Model in Rats: An In-Depth Characterization to Optimize Therapeutic Development

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Background: Cisplatin is a platinum-based chemotherapeutic agent widely used in the treatment of many cancers. However, its use is often associated with severe side effects including permanent hearing loss and cochlear inflammation. Despite its extensive use in clinical treatments, few protective strategies and no effective regenerative therapies are currently available. The development and characterization of experimental animal models are essential for understanding the mechanisms of cisplatin's ototoxicity and for developing effective treatments. This study aimed to optimize a reliable preclinical rodent model of Cisplatin-Induced Hearing Loss (CIHL) and to characterize the associated hearing impairment at functional, cellular, immunological and protein levels to efficiently develop and evaluate therapeutic compounds.

Methods: CIHL model was induced by administering a single intraperitoneal (IP) injection of cisplatin to rats (T0). Different doses of cisplatin were tested (8, 10 and 13 mg/kg). The ototoxic effects of cisplatin were evaluated and characterized using several methods: (1) Distortion Product Oto Acoustic Emission (DPOAE) and Auditory Brainstem Response (ABR) measures were conducted before and after cisplatin administration (T+3DAYS) to assess auditory function; (2) histological analyses were performed on cochleae sampled at T+3DAYS to assess hair cell (HC) loss and analyse the immune response induced by cisplatin exposure; (3) protein analyses were carried out on cochleae sampled at T+4HOURS using specific arrays to determine the signaling pathways activated by cisplatin injection.

Results: At T+3DAYS, DPOAE and ABR measures showed a dose-dependent alteration of auditory function at 10 and 13 mg/kg of cisplatin, indicating a progressive hearing loss. In contrast, a single dose of 8 mg/kg of cisplatin did not produce significant alterations in auditory function compared to controls. Immunostaining to quantify Inner and Outer Hair Cells (IHCs and OHCs) showed that the auditive deficits observed at 10 and 13 mg/kg were accompanied by a reduction in OHC density in a dose dependent manner. Histological analyses of the immune response were conducted on cochleae prepared either as flat surface or cross-sections. Ongoing analysis allowed the identification and quantification of macrophages (Iba1), T lymphocytes

(CD3) and B lymphocytes (PAX5) in the cochleae of rats exposed to cisplatin. Finally, protein analysis of cochlear tissue, targeting over 200 molecules, led to the identification and quantification of several cytokines, chemokines, growth factors and soluble receptors, most of which were classified as inflammatory and trophic factors.

Conclusions: Preclinical testing is a critical phase in drug development, underscoring the need to refine and expand tools to support in-depth characterization of in vivo models to improve the advancement of protective or regenerative therapies. In this study, 10 mg/kg of cisplatin lead to a relevant CIHL model. Its characterization from the functional to the molecular levels, including inflammatory component, yielded valuable data for the development of new therapeutic compounds.

SU119. Immune Cells and Hearing Disorders: Development of a Short In Vivo Model for Evaluating the Anti-Inflammatory Potential of Therapeutic Compounds

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Background: Following exposure to noise, ototoxic agents, or aging, immune cells, particularly macrophages, are recruited to the cochlea to contribute to tissue repair. However, their excessive activation can lead to chronic inflammation, resulting in cochlear damage and hearing loss. A better characterization of macrophage recruitment kinetics, localization and signaling pathways is essential for optimizing the therapeutic potential of anti-inflammatory compounds. This study aimed to establish a short and well characterized in vivo model of cochlear inflammation to efficiently evaluate anti-inflammatory compounds.

Methods: Cochlear inflammation was induced in mice by transtympanic (TT) injection of lipopolysaccharide (LPS) in both ears (T0). Different parameters were evaluated: the dose of LPS (5 and 10 µg/ ear) and the timepoint for histological analyses of the immune response (T+2DAYS, T+4DAYS, T+7DAYS). Auditory Brainstem Response (ABR) thresholds were measured at each timepoint to assess associated hearing impairments. Histological analyses were conducted on cochleae prepared either as flat surface or cross-sections, followed by immunolabelling to identify macrophages (Iba1), T lymphocytes (CD3) and B lymphocytes (PAX5). Immune cells quantification and qualitative assessment of their localization in the cochlea were performed to correlate with ABR outcomes.

Results: LPS injection induced a significant increase in the number of immune cells in the cochlea, as demonstrated in flat surface preparations and cross sections. Immunolabelling of macrophages, T and B lymphocytes revealed that the immune response was mainly formed of macrophages, detected in the spiral ligament and spiral ganglion neuron regions. Macrophage recruitment in the cochlea was transient and increased at T+2DAYS and T+4DAYS, but not at T+7DAYS, compared to controls. Interestingly, in mice injected with LPS, the number of macrophages correlated with both the dose of LPS and the increased ABR thresholds at T+2DAYS and T+4DAYS. At T+7DAYS both markers completely recovered.

Conclusions: In LPS cochlea, a huge recruitment of inflammatory cells was reported, thanks to histological tools, and correlated with transient hearing loss. These results validate the local use of LPS as a rapid and efficient method to induce cochlear inflammation, providing a relevant in vivo model to evaluate anti-inflammatory compounds in vivo and their effects on hearing. Immune response characterization is now being completed with protein analysis of cochleae and Complete Blood Count (CBC) tests to further characterize the inflammatory response in this model.

SU120. Can Oncomodulin Be Expressed in Macrophages Following Cochlear Damage?

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Background: Oncomodulin (OCM) is the most abundant calcium-binding protein in outer hair cells (OHCs). In mice, the absence of OCM leads to progressive hearing loss and degeneration of OHCs, indicating that OCM is essential for maintaining cochlear function. Research has shown that OCM is transiently expressed and secreted by macrophages and neutrophils in response to injuries in the eye and spinal cord. The released OCM promotes nerve regrowth and repair for retinal ganglion cells and dorsal root ganglia. However, it remains unclear whether OCM can also be expressed in macrophages and neutrophils following injuries in the cochlea, just as in damaged eye and spinal cord. In the current study, we aim to investigate OCM expression in macrophages after cochlear damage using a new *Ocm*-KO/*lacZ* reporter mouse model (*Ocm* *tm1b*), which replaces *Ocm* translation with the expression of β -galactosidase (β -gal).

Methods: We created an *Ocm*-KO mouse model (*Ocm* *tm1a*) based on the ‘KO-first, conditional-ready’ strategy. *Ocm* *tm1a* mice were then crossed with CMV-Cre mice, which can delete the neo cassette and exon 3 of the *Ocm* *tm1a* allele to generate a *lacZ*-tagged global KO allele (*Ocm* *tm1b*). To confirm the absence of OCM in *Ocm**tm1b*/*tm1b* mice, we collected cochleae from WT and *Ocm**tm1b*/*tm1b* mice for immunofluorescence (IF) and Western blot with an antibody for OCM. To validate β -gal protein expression in the OHCs of *Ocm**tm1b*/*tm1b* mice, we performed X-gal staining in the cochleae from WT and *Ocm**tm1b*/*tm1b* mice. Additionally, we performed IF with antibodies for β -gal and OHC marker Prestin. To investigate whether OCM is expressed in macrophages within damaged cochlea, we administered a single intravenous dose of 16 mg/kg cisplatin to *Ocm**tm1b*/*tm1b* mice. One and two days after injection, we collected cochleae to perform IF with antibodies for β -gal and macrophage marker IBA1.

Results: OCM protein expression was not detected in *Ocm**tm1b*/*tm1b* cochleae, but it was present in WT cochleae. Additionally, β -gal protein expression was observed in the OHCs of *Ocm**tm1b*/*tm1b* cochleae but not in those of WT cochleae. There was no detectable β -gal staining in macrophages labeled with anti-IBA1 in untreated *Ocm**tm1b*/*tm1b* cochleae. However, a few β -gal and IBA1 positive cells were found in the stria vascularis of the *Ocm**tm1b*/*tm1b* cochleae collected 1 day after cisplatin injection.

Conclusions: These results show that OCM can be transiently expressed in macrophages in damaged cochlea. Since cisplatin damages many tissues and cells, it remains unclear whether the β -gal and IBA1 positive cells originate from resident macrophages in the cochlea or circulating

monocytes from the blood due to cisplatin-induced damage in other tissues. To address this uncertainty, we will use noise exposure to induce tissue damage exclusively in the ear. (Work supported by the Knowles Leadership Fund and NIH R56DC020542 to JZ).

SU121. Spatial Organization of Cochlear and Vestibular Lymphatics in the Mouse Model

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Background: The lymphatic system circulates interstitial fluid and immune cells to return to the bloodstream, playing a critical role in tissue fluid homeostasis and the immune surveillance system. The system consists of lymphatic vessels (lymphatics) and lymph nodes. For many years, the nervous system has been considered devoid of a lymphatic vascular system. Recent discoveries of the lymphatic vascular systems draining the brain and cerebrospinal fluid have brought a paradigm shift. Lymphatics of some sensory systems, like the visual systems, have also been described. However, the lymphatic vascular system for the sensory organs of hearing (cochlea) and balance (vestibular system) has never been described. The current study is aimed at exploring the possibility of lymphatic vessels in the cochlea and vestibular systems.

Methods: 8-12-week-old mice were used to identify the cochlear and vestibular lymphatic vessels. Thy1+/YFP and PECAM1-EGFP mice on C57B6 background were used to differentiate lymphatics from SGNs and blood vessels, respectively. The cochlea and vestibule, fixed with 4% PFA, were decalcified with 0.12M EDTA and cryopreserved with increasing concentration (10%-30%) of sucrose. 30-100µm thick cochlear and vestibular sections and cochlear whole mounts were labeled with antibodies against lymphatic markers (PDPN, Lyve1, VEGFR3), anti-GFP (to amplify Prox1-EGFP signal), and Anti-myosin VIIa (hair cell marker) antibodies and imaged using a confocal microscope. Whole cochlea and vestibule samples labeled with markers of lymphatics were cleared using the CUBIC tissue clearing protocol.

Results: A robust lymphatic vascular network, labeled with markers of lymphatics, was observed in the cochlea and vestibular systems. Within the cochlea, lymphatics are observed in the spiral ganglia, stria vascularis, spiral ligament, Reissner's membrane, along the margins of the scala tympani and scala vestibuli, modiolus, and underneath the hair cells. PDPN+ lymphatics could be distinguished from Thy1+ SGN fibers and PECAM1+ blood vessels. We have observed the lymphatic vessels in different parts of the vestibular system, including the saccule, utricle, vestibule, and semicircular canals. Expectedly, both cochlear and vestibular lymphatics are adjacent to the cochlear blood vessels and located in strategic locations suitable for draining major sensory structures, and interstitial (perilymph and endolymph) fluids.

Conclusions: Our results suggest the presence of a cochlear and vestibular lymphatic network in adult mice. Further investigations using different lymphatic markers are necessary for anatomical and molecular characterization of cochlear lymphatics.

SU122. Role of the Cochlear ‘Immune Surveillance System’ in the Foreign Body Response Following Cochlear Implantation

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Background: The inflammatory foreign body response (FBR) following cochlear implantation (CI) has the potential to negatively impact CI outcomes, including increased electrode impedances, accelerated loss of residual acoustic hearing, reduced battery life, and, rarely, implant extrusion. Immune cells contribute to the FBR post-CI. Immune cells are trafficked to the tissue via blood vessels and return to the bloodstream via the lymphatic vessels, forming the ‘immune surveillance system’. This study aims to investigate the role of immune cell trafficking via cochlear blood vessels and the recently discovered cochlear lymphatic network in the FBR post-CI.

Methods: To investigate the immune cell trafficking through cochlear blood vessels and lymphatics, we used five mouse strains: CX3CR1+/GFP (reporter for mononuclear phagocytes/MNPs), CCR2+/RFP CX3CR1+/GFP (reporter for blood-derived/ hematogenous/ infiltrated/extravasated MNPs), Prox1tdTomato-CD11cGFP (lymphatic-dendritic cells dual reporter), Prox1tdTomato-CX3CR1+/GFP (lymphatic-mononuclear phagocyte dual reporter), and Prox1tdTomato-PECAM1GFP (lymphatic-blood vessel dual reporter). The left ears of 10-12-week-old mouse models were implanted with a standard non-functional CI. Following the euthanasia of mice at 28 days post-operatively, harvested cochleae fixed with 4% PFA were processed and cryosectioned at 30µm parallel to the mid-modiolar plane. Sections labeled with antibodies against appropriate markers were used to identify different cell types: CD45, CD3e, MHCII, anti-GFP, Lyve1, VEGFR3, and PDPN. Using IMARIS image analysis software, the density of MNPs, infiltrating immune cells, CX3CR1+MHCII+ (APC), CX3CR1-CD45+ (non-MNP immune cells), CX3CR1-CD45+ CD3e+ (T lymphocytes), and CD11cGFP+ (dendritic cells) was calculated. The volume of blood vessels (PECAM1+) and lymphatic vessels (Prox1tdTomato+, PDPN+VEGFR3+Lyve1+) was measured within the scala tympani of the base of the cochlea, and the density of the blood vessels and lymphatic vessels in the scala tympani of the cochlea was quantified. Intralymphatic immune cell density was quantified, creating masks for lymphatic vessels and the immune cells.

Results: Following CI, blood vessels proliferate into the cochlea, trafficking blood-derived immune cells into the cochlea. Cochlear implantation also leads to lymphatic vessel proliferation into the cochlea, trafficking immune cells. Recruitment of MHCII+APCs, dendritic cells, and T lymphocytes suggests an activation of the adaptive immune system.

Conclusions: Our data indicate that following cochlear implantation, vascular proliferation is associated with a two-way traffic of immune cells to (via blood vessels) and away (via lymphatics) from the cochlea. This supports the involvement of the cochlear ‘immune surveillance system’ in FBR post-CI.

SU123. Diagnostic Yield of Whole-Genome Sequencing in Hearing Impairment: A Five-Year Study in a Large German Cohort

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Background: Hereditary hearing loss is a genetically and clinically heterogeneous condition with variable age of onset, severity, and progression. Genetic testing has shifted from targeted panels and exome sequencing toward whole-genome sequencing (WGS), which enables more comprehensive variant detection and deeper insights into the genetic spectrum of hearing loss. This study aimed to retrospectively evaluate five years of whole-genome sequencing for hereditary hearing loss at a tertiary care center in Southwest Germany by assessing diagnostic yield, the spectrum of causative genes, and factors influencing the likelihood of a molecular diagnosis.

Methods: 383 index probands with suspected hereditary hearing loss were identified, who underwent WGS between April 2020 and July 2025. Phenotypic characterization was performed by reviewing the electronic medical records and the institutional audiometric database, collecting information on family history, age of onset, degree and laterality of hearing loss. These phenotypic variables were additionally complemented by genetically derived estimates of ethnicity and consanguinity. Logistic regression was applied to estimate the effects of predictors on diagnostic outcome, with significance levels adjusted for multiple testing.

Results: The age at genetic testing ranged from 0.2 to 84.6 years (mean $\pm$ SD: 33.8 ± 23.1 years). A postlingual onset of hearing loss was present in 53.0% of probands, while 31.3% presented with congenital and 14.9% with early-onset (pre- or perilingual) hearing loss. A positive family history of hearing loss was reported in about half of the probands (53.0%). Of the 383 probands analyzed, a genetic diagnosis was obtained in 106 cases (27.7%). Among the solved cases, autosomal-recessive inheritance predominated (63.2%), followed by autosomal-dominant inheritance (30.2%). The underlying variants were distributed across 37 genes, with a marked predominance of GJB2, which accounted for 35% of all solved cases. Six additional genes contributed 24.5% of diagnoses, including MYO6 (5.7%) and COCH, MYO15A, MYO7A, PTPRQ, and TMPRSS3 (all 3.8%). The remaining 40.5% of genetically diagnosed probands were linked to variants in 30 other genes. In logistic regression analysis, age at onset of hearing loss, positive family history, and degree of hearing loss emerged as significant predictors of diagnostic outcome. Pairwise contrasts of estimated marginal means further indicated higher predicted diagnostic rates in probands with a positive family history compared to those without, and in congenital or early-onset hearing loss compared to postlingual onset.

Conclusions: In this large German cohort, genetic testing for hereditary hearing loss revealed a broad spectrum of causative genes and provided an overview of the genetic landscape in the population, highlighting its clinical relevance. Certain clinical factors emerged as predictors of diagnostic outcome, emphasizing the value of detailed phenotyping for genetic diagnostics in hearing loss.

SU124. Inner Ear Malformations in Patients With Hearing Loss — Exploring the Genetic Architecture

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Background: Inner ear malformations (IEMs) can occur in isolation or as part of genetically determined syndromes. Enlarged vestibular aqueduct (EVA) is the most frequently observed IEM, often accompanied by incomplete partition type 2 of the cochlea and a dilated vestibule. SLC26A4 is the major gene associated with IEMs, with 50% of EVA/IP2 patients carrying either two pathogenic SLC26A4 variants or one SLC26A4 pathogenic variant together with the CEVA haplotype. The genetic background of other IEMs is more diverse and remains poorly understood.

Methods: DNA was isolated from blood samples of 40 hearing loss (HL) patients with bilateral IEMs. Sequencing was performed using a custom multigene panel (237 or 263 genes).

Results: Genetic testing identified the cause of IEMs in 67.5% (27/40) of individuals. Among EVA/IP2 patients, six carried two pathogenic variants in SLC26A4, while another six had one SLC26A4 pathogenic variant together with the CEVA haplotype. The genetic cause still remains unknown in six EVA/IP2 patients. In patients with other IEMs, pathogenic variants were identified in POU3F4 as well as genes associated with CHARGE (CHD7), BOR (EYA1, SIX1), Waardenburg (SOX10), and Kabuki (KMT2D) syndromes. Additionally, a novel pathogenic variant in HOXA2 was identified in a single patient.

Conclusions: Mutations in SLC26A4 and the CEVA haplotype account for more than half of EVA/IP2 cases. Patients with other IEMs are frequently diagnosed with syndromic forms of HL. Further research is needed to identify pathogenic variants in noncoding regions of known HL genes or in novel genes.

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SU125. Immune Modulation of Zfp719 Dependent Hearing Loss

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Background: Hearing loss affects ~20% of the population, with sensorineural forms predominating. Causes include genetics, environmental factors, trauma, and aging. Adult-onset hearing loss not only impacts individual well-being but also carries substantial socioeconomic implications. Our research seeks to elucidate unexplained genetic underpinnings of adult-onset hearing loss, focusing on rare coding variants within established and undiscovered hearing loss genes. Taking advantage of a genome first approach in the Penn Medicine BioBank and functional studies in mice we identified ZNF175, and its mouse orthologue Zfp719, as a novel adult onset hearing loss gene. ZNF175/Zfp719 is a member of a large family of Krüppel-

associated box zinc-finger proteins that bind DNA and silence target gene expression, including transposable elements and host genes, through the formation of heterochromatin.

Methods: Genomic and bioinformatic analysis of snRNA-seq datasets, quantitative spatial transcriptomics, a flexible genetic system in mice were used to identify the gene targets of Zfp719 mediated heterochromatin silencing and determine the pathogenic consequences of their misregulation on calcium ion homeostasis, ribbon synapse integrity, hair cell viability, and innate immunity.

Results: Zfp719 mutant mice exhibited progressive synaptopathy, hair cell degeneration and hearing loss, with severity increasing in both an age- and gene dosage- dependent manner. Tissue-resident macrophages infiltrated the cochlea before overt signs of cellular damage and persisted as pathology advanced along the cochlear duct from base to apex. Selective deletion of Zfp719 in hair cells, but not in spiral ganglion neurons, recapitulated these cochlear defects, including macrophage activation. Strikingly, pharmacological depletion or genetic disruption of macrophages accelerated cochlear pathology in Zfp719 mutants, revealing a protective role in delaying both the onset and severity of hearing loss. Single-nucleus transcriptional profiling identified Gm15083, a long non-coding RNA, as one of the most upregulated genes in Zfp719 mutants. Gm15083 overlaps with Atp2b2 on chromosome 6 and is transcribed in antisense orientation, raising the possibility that derepression of Gm15083 interferes with Atp2b2 transcription. Atp2b2 encodes plasma membrane calcium transporting ATPase 2 (PMCA2), a crucial efflux pump required to maintain calcium homeostasis in outer hair cells. Consistent with this model, Atp2b2 decreased and Gm15083 increased in Zfp719 mutants outer hair cells. Moreover, Atp2b2 haploinsufficiency closely phenocopied the Zfp719 mutant phenotype, implicating disrupted calcium homeostasis as a primary driver of hearing loss. Ongoing experiments are testing whether loss of Gm15083 transcription restores Atp2b2 expression and auditory function in Zfp719 mutants.

Conclusions: Zfp719 is required in cochlear hair cells for normal auditory function, and its loss triggers gene dosage-dependent degenerative defects. Cochlear macrophages act protectively in this context. The concordant changes in Gm15083 and Atp2b2 expression indicate that disrupted calcium homeostasis is a principal consequence of Zfp719 deficiency. Given the multifactorial etiology of adult-onset hearing loss, our findings may help inform a broader understanding of the condition.

SU126. USH2A Variants as a Significant Genetic Factor an Mild to Moderate Hearing Loss

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Background: The most common genetic cause of mild to moderate hearing loss (HL) is STRC copy number variants (CNVs). Many patients with mild to moderate HL require further genetic testing due to the lack of molecular diagnosis. Literature indicates that, besides STRC, USH2A, MPZL2, and OTOG may also be responsible for mild to moderate HL. This study aimed to identify the genetic basis of isolated mild to moderate HL in patients without STRC CNVs.

Methods: The study included 47 patients with isolated mild to moderate HL without detected STRC CNVs or with one identified heterozygous STRC CNV. Genomic DNA was isolated from peripheral blood and NGS with a custom panel of HL-related genes (n=237 or 263) was performed. Selected variants were confirmed by Sanger sequencing.

Results: NGS revealed the genetic cause of HL in 51% (24/47) of patients. Among these, 25% (6/24) of causative variants were found in USH2A, linked to Usher syndrome. In 17% (4/24) of variants, mutations in OTOG were considered as the cause of HL. The remaining causative variants were detected in: ADGRV1, GATA3, MPZL2, OTOA, POU3F4, PTPRQ, REST, STRC, TBC1D24, and WFS1.

Conclusions: NGS revealed that, in addition to STRC, a significant proportion of patients with mild to moderate HL have causative variants in USH2A. Patients with USH2A pathogenic variants are likely to develop Usher syndrome type 2A, which includes both HL and retinitis pigmentosa. Identifying the genetic cause is crucial for providing personalized care from an early age.

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SU127. Identification of Pathogenic Synonymous SLC26A4 Variants

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Background: Variants in SLC26A4 are a common cause of autosomal recessive hearing loss (HL) associated with an enlarged vestibular aqueduct (DFNB4/non-syndromic enlarged vestibular aqueduct (EVA)) and Pendred syndrome (HL and thyroid goiter). In ~15-30% of cases, biallelic variants SLC26A4 are not identified, suggesting the presence of an unidentified second pathogenic variant. We hypothesized that in some unresolved cases, the second variant is a splice-altering synonymous change.

Methods: A prospective study identified candidate variants in persons tested using the latest version of our comprehensive genetic testing platform (OtoSCOPE®). Variants were filtered and prioritized based on minor allele frequency, in silico predictions, family history, and clinical correlation.

We also performed a retrospective analysis of 199 persons with a single SLC26A4 pathogenic/likely pathogenic variant, a phenotype potentially consistent with SLC26A4-related HL, and no genetic diagnosis using the current version of the bioinformatics workflow, which assesses synonymous variants to identify synonymous variants of interest that were being previously filtered out.

The functional impact of candidate variants was assessed using minigene splicing assays. Patient DNA containing the relevant SLC26A4 exon and a portion of the flanking intronic regions was

subcloned into a pre-constructed pET01 Exontrap vector encoding 5' and 3' exons separated by a multiple cloning site. Vectors were transfected into COS-7 cells. RNA harvest was performed 36-48 hours post-transfection. Changes in splicing were assessed using gel electrophoresis and Sanger sequencing.

Results: From the prospective analysis of patients, we identified a 2-year-old male with congenital, moderate-to-severe bilateral sensorineural HL and bilateral EVA. He carries the novel synonymous variant, NM_000441.2:c.600G > A, p.(Gln200=) together with a previously reported pathogenic variant, NM_000441.2:c.1151A > G, p.(Glu384Gly). The minigene splicing assay indicated that the c.600G > A variant results in skipping of exon 5, generating a frameshift.

From the retrospective analysis, we identified a 7-year-old female with sensorineural HL diagnosed at the age of 5, EVA, and a brother with HL. She carries the rare synonymous variant, NM_000441.2:c.1206G > A, p.(Val402=) variant together with the pathogenic variant, NM_000441.2:c.2284G > T, p.(Glu762*). The minigene splicing assay indicated that the c.1206G > A creates a cryptic splice acceptor 58 bases into exon 10 and a resulting frameshift.

Conclusions: This work highlights the importance of evaluating persons for splice-altering synonymous variants to identify pathogenic variants in autosomal recessive conditions. However, there are still many undiagnosed cases of presumed SLC26A4-related HL, highlighting the need for other methods of detection to resolve the challenge of missing heritability.

SU128. Identification of a Novel Founder EYA4 Structural Variant in North America

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Background: Autosomal dominant nonsyndromic hearing loss (NSHL) is a genetically heterogeneous disorder. Copy-number and structural variants (SVs) in EYA4 are a rare cause of hearing loss at the DFNA10 locus. We aimed to determine the genetic etiology of hearing loss in a series of families with autosomal dominant NSHL.

Methods: Retrospective review of patients referred for targeted genomic enrichment and massively parallel sequencing using the OtoSCOPE® panel, which covers all exons and flanking intronic sequences of known genes associated with NSHL. Genetic results and available phenotypic data were reviewed by a multidisciplinary team to assess variant pathogenicity. Haplotypes were reconstructed using short tandem repeat analysis (STRP) and variants in EYA4 and surrounding regions.

Results: A novel pathogenic SV in EYA4 was detected in five North American probands of European descent with family history suggestive of autosomal dominant NSHL, which involves a 5.8kb deletion of the C-terminal exons 19 and 20, and insertion of a 76-bp inverted downstream sequence. Segregation analysis revealed co-segregation with the hearing loss phenotype in 2 affected relatives. This SV is the most frequently detected pathogenic variant in

EYA4 in the OtoSCOPE® cohort, accounting for 5 of 25 (20%) of DFNA10 diagnoses to date and 5 of 15 (33%) diagnoses in probands of European ancestry. STRP haplotype analysis demonstrated an 857 kB region surrounding EYA4 in linkage disequilibrium, shared among all probands and affected family members.

Conclusions: This finding expands the mutational spectrum of EYA4-associated deafness, with identification of a novel SV as the most common pathogenic DFNA10 variant in a large hearing loss cohort. The complexity of this SV, coupled with haplotype reconstruction, strongly suggests a founder effect rather than a recurrent event. These data highlight the importance of critical analysis of high-quality next-generation sequencing in the assessment of complex SVs.

SU129. Genetic Insights Into Sensorineural Hearing Loss: Identifying Variants in Hispanic Children

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Background: Sensorineural hearing loss (SNHL) affects approximately 1 to 3 per 1,000 live births, with over half of congenital cases attributed to genetic causes. While major strides have been made in understanding the genetic basis of SNHL particularly in European and Asian populations, Hispanic/Latino populations remain vastly underrepresented in genetic studies and testing, limiting equitable access to diagnostics and potential interventions.

Methods: To address this disparity, we enrolled Hispanic-admixed pediatric patients with confirmed SNHL and their family members through collaborations across four sites in three countries. Saliva samples were collected using Oragene DNA kits, and comprehensive clinical information — including family history, audiologic evaluations, surgical reports, and imaging — was reviewed. Genomic DNA was isolated from saliva and submitted to either exome sequencing (n=26) at the University of Washington Northwest Genomics Core or whole-genome sequencing (WGS; n=25) at the University of Colorado Genomics Core. The generated sequence data were analyzed using a custom pipeline incorporating ANNOVAR and gnomAD minor allele frequency (MAF) filters. Prioritized variants were further assessed using functional prediction tools for single nucleotide variants and indels. Copy number or structural variants were called

using multiple tools including Manta, Strelka, CNVkit and FreeBayes. Identified pathogenic and putatively functional variants were Sanger-sequenced in the proband and other family members.

Results: Preliminary analyses based on exome sequence data identified several pathogenic or likely pathogenic variants in known hearing loss genes, including population-specific alleles such as GJB2 c.19C > T (p.Gln7*) that was previously identified in Ecuador and GJB2 c.131G > A (p.Trp44*) in Guatemala. Early findings suggest that allelic spectra defined by genes, variants, and MAF differ across recruitment sites and in comparison, to non-Hispanic populations reported in the literature. Furthermore, to date our cohort shows a higher proportion of syndromic relative to nonsyndromic HL, a trend supported by the few existing reports on Hispanic populations.

Conclusions: These results underscore both the shared and unique genetic architecture of SNHL in Hispanic groups. Importantly, the diagnostic yield from this study supports the inclusion of genetic testing in hearing care protocols for Hispanic children, with implications for early intervention and eligibility for emerging gene therapies. Future directions include analyzing additional WGS datasets, expanding recruitment across diverse Hispanic regions, optimizing variant interpretation, and disseminating findings to guide equitable implementation of precision medicine in otolaryngology.

SU130. Dysregulation of Extracellular Matrix and Inner Ear Components Underlying the Hypoplastic Endotype of Ménière's Disease: Role of ADAMTS18

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Background: Meniere Disease (MD) is a chronic inner ear disorder characterized by fluctuating sensorineural hearing loss (SNHL), tinnitus, and episodic vertigo, with variable severity and progression. Its etiology remains unclear, with autoimmune, viral, allergic, vascular, developmental, and genetic factors under investigation. Previous studies of human temporal bone consistently identified abnormalities of the endolymphatic sac in MD, correlating with distinct clinical pictures. In ~30% of MD cases, the sac remains developmentally arrested or hypoplastic (MD-hp) with patients showing early-onset disease, male predominance, bilateral symptoms, temporal bone malformations, and familial clustering of MD or SNHL. These features point to a genetic cause for this endotype, and our aim was to define its underlying basis.

Methods: MD patients were recruited following diagnostic guidelines, and the MD-hp endotype was identified using a surrogate radiodiagnostic marker in CT. Whole-exome sequencing was performed from saliva-derived DNA. Enrichment of rare SNPs and InDels (AF < 0.05) with predicted high/moderate impact were analyzed through gene burden analysis against general population databases. Structural variants were also called and prioritized by high constraint or pathogenic prediction. Functional analysis was performed with candidate genes revealing

implicated biological pathways. Candidate gene expression was further evaluated by immunohistochemistry in human and mouse temporal bone across different development stages.

Results: Our final cohort consisted of 42 MD-hp individuals. Candidate genes included 94 genes enriched in potentially causal variants. Functional enrichment analysis revealed biological pathways related to extracellular matrix formation and maintenance, including bone matrix development and non-syndromic hearing impairment. Among these, ADAMTS18 emerged as the strongest candidate with 35.7% of MD-hp patients carrying 15 distinct variants in this gene. Immunolabeling confirmed ADAMTS18 expression in human and mouse temporal bone during embryonic and early postnatal stages, both surrounding sensory epithelia and within the maturing cartilage destined to form the otic capsule.

Conclusions: Our findings support a strong genetic contribution to the MD-hp endotype, with an accumulation of variants in genes involved in inner ear structures and in the ossification of the surrounding temporal bone. In particular, ADAMTS18 harbors variants predominantly in MD-hp patients, and its disruption may underlie abnormal development of these structures during embryonic and early postnatal stages. Genetic delineation of this endotype provides new insight into disease mechanisms, supports stratification of MD patients into biologically meaningful subgroups, and may ultimately guide precision approaches for diagnosis and therapy.

SU131. Uncovering the Genetic Relationship Between Dementia and Age-Related Hearing Loss

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Background: Hearing loss is an important modifiable midlife risk factor for dementia. Recent studies have investigated the genetic relationship between dementia and age-related hearing loss (ARHL) with conflicting results. These analyses have conveniently treated ARHL and dementia as monolithic conditions, when in reality both encompass distinct pathophysiological processes. We have previously shown that genes linked to frontotemporal dementia may play a role in the metabolic subtype of ARHL (MHL), but not other ARHL subtypes. Other emerging evidence also suggests that genetic risk for hearing loss associates with specific brain structural changes, though these findings are typically based on broad hearing phenotypes. Therefore, we sought to employ a comprehensive phenotyping strategy using audiological measures and brain magnetic resonance imaging (MRI) data to examine whether genetic variants that we have found to be associated with MHL also affect brain structure, potentially providing mechanistic insight into the genetic relationship between ARHL and dementia.

Methods: Through the Canadian Longitudinal Study on Aging (CLSA), we have access to high-quality genotype and audiological data for 17,531 older adults. Estimates of MHL were calculated from audiograms of European-descent participants and subjected to genome-wide association study (GWAS) analyses to identify genetic variants associated with the MHL subtype. MRI GWAS data were previously generated by the UK Biobank (n=33,000) and accessed online. A phenome-wide association study (PheWAS) was performed to identify brain MRI traits that shared risk loci with MHL. Genetic colocalization was then performed to confirm that the same causal genetic variants were underlying these shared signals.

Results: PheWAS identified 7 MRI phenotypes with the strongest evidence of association ($P < 2 \times 10^{-5}$) with MHL risk variants. Colocalization analysis of these prioritized MRI-MHL-variant triplets identified strong evidence (92.8% posterior probability) for a shared genetic variant (rs4413512) in ARHGEF28 underlying both MHL and white matter microstructure in the retrolenticular segment of the internal capsule, a white matter pathway transmitting sensory information. This finding suggests that genetic variants associated with MHL may also affect brain white matter integrity, though the functional significance requires further investigation.

Conclusions: This exploratory study suggests (1) the utility of highly specific, objective phenotyping in genetic analyses of complex traits, and (2) preliminary evidence for shared genetic architecture between ARHL and brain white matter. The colocalization of an intronic variant in ARHGEF28 with white matter microstructure measures in the internal capsule may reflect the gene's dual role in regulating both cytoskeletal organization and RNA metabolism, two processes essential for maintaining axonal integrity and myelination. Looking ahead, future research expanding to additional dementia endophenotypes and ARHL subtypes will help clarify the role of hearing loss as a modifiable midlife risk factor for dementia, guiding both clinical decision-making and research on the hearing-dementia association.

SU132. Optimizing Genetic Testing Referrals for Pediatric Hearing Loss

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Background: Although the benefits of genetic testing in pediatric hearing loss are well established, referral rates for genetic testing remain low within otology and audiology practices. This study examined referral rates, referral pathways, and appointment completion for genetic evaluation in an ethnically diverse pediatric cohort before and after implementation of a quality improvement (QI) protocol.

Methods: In phase 1 (Jan-Aug 2023), chart reviews were conducted for 86 patients with hearing loss to determine if referrals to genetics were made. Data collected included demographics, referral modality (progress note documentation, routed note to genetics, or direct referral order) and genetics appointment status. In Phase 2 (Sep-Dec 2023), a standardized referral protocol was implemented requiring all newly diagnosed patients to be referred through one of three pathways (visit summary note, routed note, direct referral order). Providers received a brief training and reminder cards. Chart reviews were then conducted on 29 additional patients.

Results: In phase 1, 27.9% had a referral documented. Referrals were made by otologists (15.1%), audiologists (7%), or both (5.8%). A routed note occurred in 4.7% of cases, and a direct referral order in 15.1%. Among referred patients, 54.2% did not schedule an appointment, 20.8% had pending appointments, and 25% completed an appointment.

In phase 2, referral documentation increased to 55.2% of patients. Referrals were made by audiologists (31%), otologists (6.9%), or both (17.2%). A referral order was placed in 41.4% of patients, and 31% had a routed note. Appointment completion improved to 62.5% compared to 25% in Phase 1.

Conclusions: Genetic testing referrals remain underutilized but improved after implementing a standardized protocol. These findings emphasize the need to identify barriers in referral

pathways and provide ongoing provider training. Quality improvement projects offer a promising approach to optimize genetic evaluations and enhance precision care for pediatric hearing loss patients.

SU133. Estradiol Regulation of Cochlear Gene Expression in Female Mice

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Background: Sex is a biological factor that influences auditory function and disease susceptibility. Our recent study showed that sex affects cochlear gene expression, but the mechanisms regulating this sex-dependent expression remain unclear. Estrogen, a major female hormone, is known to modulate gene expression in many non-cochlear organs and tissues. This study aimed to investigate the effects of estradiol treatment on cochlear gene expression and to assess the functional implications of estrogen-responsive genes in the cochlea.

Methods: Young, healthy female CBA/CaJ mice were used. To eliminate gonadal hormone production, the mice underwent ovariectomy (OVX). OVX mice were then injected daily with 17 β -estradiol at a dose of 3 μ g dissolved in sesame oil for three consecutive days, while control OVX mice received equivalent volumes of sesame oil alone. At the end of treatment, mice were sacrificed and their cochleae were collected. Bulk RNA-sequencing was performed to compare cochlear transcriptomes between estradiol-treated and control mice. Differentially expressed genes (DEGs) were identified, and their functional associations were analyzed using multiple bioinformatics tools.

Results: Using the criteria of adjusted p-value < 0.05 and log₂ fold change (FC) > 0.2, we identified 366 DEGs (165 upregulated and 201 downregulated). Of these, 98.4% were protein-coding genes, while the remainder were non-coding RNAs and pseudogenes. Most DEGs exhibited only mild expression changes with the log₂ FC < 1. The five most upregulated genes with log₂ FC > 1 were *Prl*, *Mgl2*, *Lars2*, *Siglec1*, and *Clec10a*. The three most downregulated genes were *Hspa1a*, *Kcnj11*, and *Hspa1b*. While these genes have diverse functions, many are involved in immune and stress responses. Gene Set Enrichment Analysis (GSEA) revealed distinct functional signatures for up- and downregulated genes. Among the upregulated genes, oxidative phosphorylation and fatty acid metabolism showed the highest normalized enrichment scores with adjusted p-value < 0.05. In contrast, downregulated genes were enriched for TGF- β (transforming growth factor β) signaling. This pathway regulates multiple processes, including development, immune function, and tissue repair. Additional biological processes and cell signaling identified through the Database for Annotation, Visualization, and Integrated Discovery (DAVID) included Wnt signaling, stress responses, transcriptional regulation, and biological rhythms.

Conclusions: Our study demonstrates that estrogen modulates the expression of cochlear genes with diverse functions, particularly those involved in energy metabolism and stress regulation. These findings enhance our understanding of sex-related differences in cochlear homeostasis and susceptibility to auditory disease and dysfunction.

SU134. Cellular and Transcriptional Heterogeneity Due to NF2 Deficiency: Insights From Human iPSCS

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Background: Loss-of-function mutations in NF2 give rise to NF2-related schwannomatosis (NF2-SWN), a rare autosomal dominant disorder characterized by the development of benign tumors of the nervous system, most notably bilateral vestibular schwannomas. Clinically, NF2-associated vestibular schwannomas (VS) present with a spectrum of symptoms including sensorineural hearing loss (SNHL), tinnitus, vertigo, and balance dysfunction.

Methods: Herein, we employed sex- and age-matched human iPSC lines, including CRISPR/Cas9-mediated isogenic NF2^{-/-} iPSC derived from healthy controls, as well as iPSC obtained from a patient with NF2 VS. To further delineate the effects of NF2 loss, we implemented a Tet-On NF2 inducible system in NF2-deficient cells to establish transcriptomic and phenotypic contrast between NF2 loss- and gain-of-function state. Through comprehensive transcriptome analysis, we traced molecular footprints and phenotypic features altered by NF2 deficiency in SCP state.

Results: Multimodal comprehensive transcriptome analysis revealed genes that are perturbed and directly involved in cellular heterogeneity due to biallelic loss of NF2. In addition, we consistently observed elevated expression of collagen-containing ECM genes and hyperactivation of PI3K/AKT pathways that are critically involved in cell survival, proliferation, and apoptosis. Thus, we investigated the cytotoxicity and evaluated the synergistic potential of the PI3K/AKT pathway inhibitors HCQ and QC in NF2-deficient SCP lines.

Conclusions: Our findings offer new insights into tumorigenesis and cellular heterogeneity in NF2-associated VS and highlight the value of patient-derived iPSCs and their derivatives as robust in vitro platforms for drug screening and therapeutic development. Ultimately, by repurposing FDA-approved drugs, HCQ and QC, we propose a more expeditious and safer approach to developing therapeutic strategies for NF2-associated VS.

SU135. The Role of a Conserved Extracellular Loop in TMC1 Gating

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Background: The transmembrane channel-like (TMC) family is an ancient group of proteins conserved across eukaryotes. During evolution, the mechanosensory channels TMC1 and TMC2 first appeared in the vertebrate lineage; they are expressed perhaps exclusively in vertebrate hair cells and seem to be highly specialized to mediate hearing and balance. In mammals, TMC1 appears to have undergone further specialization to function in its primary role in adult auditory transduction. Yet the molecular mechanism by which force opens TMC1 channels is not well understood. Here, we investigate the role of a conserved extracellular loop between transmembrane helices 1 and 2 (the 1-2 loop) that may contribute to channel gating.

Methods: We first used evolutionary analysis to find residues in TMC1 that were positively selected for and may underly its functional adaptations. We then used structural modeling to predict how these regions may affect overall structure and transduction complex binding partners. To assess functional consequences, we then selectively mutated individual amino acids in TMC1, constructed AAV vectors carrying the mutant sequence, injected AAVs into the cochleas of

Tmc1/2 null mice at P1, and recorded transduction currents from cochlear hair cells in vitro at P8-15.

Results: Evolutionary analyses revealed residues in the TMC1 1-2 loop that were positively selected in mammals. Structural modeling suggested that this loop arches over the extracellular side of the pore, approaching both the auxiliary subunit TM1E and the pore-lining helices TM4 and TM6. Mutating two conserved aromatic residues shifted channel activation towards the closed state without affecting slope, suggesting that these residues participate in biasing the channel to be open part of the time at rest.

Conclusions: A combination of evolutionary probing, structure analysis, and electrophysiology suggest the 1-2 loop has a specialized role in mammalian hearing. Targeted mutagenesis influences channel activation and in following, this loop contains several residues linked to hereditary deafness. Integration of evolutionary, structural and physiological studies provides a holistic platform for investigating residues of functional importance in sensory transduction channels.

SU136. Estradiol Increases the Vulnerability to Hidden Hearing Loss in Female Mice Lacking GluA3 Ampar Subunits

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Background: The GluA3 AMPAR subunit is essential for cochlear ribbon synapse integrity. We previously showed that female GluA3-knockout (GluA3KO) mice develop an early-onset hidden hearing loss (synaptopathy) when raised in ambient sound levels, showing reduced ABR wave-1 amplitude, significant synapse loss and afferent terminals swelling by 5-weeks of age (Pal et al., 2025). This phenotype was absent in age-matched male GluA3KO mice (Rutherford et al., 2023). However, both female and male GluA3KO mice had higher GluA4:GluA2 ratio suggesting an increased in Ca⁺⁺-permeable AMPARs (cp-AMPA) at cochlear-ribbon synapses. This sex specific vulnerability suggests a hormone mediated mechanism. Among gonad-sex hormones, estradiol is thought to be otoprotective. However, it can also potentiate high-conductance synaptic cp-AMPA in the female brain (Jain and Woolley, 2023). We therefore hypothesized that in the absence of GluA3, estradiol could increase the hearing vulnerability to ambient sound, particularly affecting females.

Methods: To test the role of gonad sex hormones in hearing vulnerability, we performed bilateral gonadectomies (ovariectomy in females and orchidectomy in males) or sham surgery in female and male GluA3KO mice at postnatal day 22 (P22). Naïve wild-type (WT) female and male mice served as additional controls. We conducted a longitudinal assessment of cochlear function by measuring ABR clicks and pure-tones thresholds, and wave-1 amplitude and latency

in all groups at P20, P35, P65, and P90. Blood collection was performed at P90 to measure sex hormones levels. The structure of cochlear-ribbon synapses will be analyzed ultrastructurally.

Results: Only sham-operated female GluA3KO mice showed reduced wave-1 amplitude at P35 compared to WT controls, confirming early-onset of hidden hearing loss. Interestingly, ovariectomized female GluA3KO mice showed wave-1 amplitudes comparable to WT mice at P35. This protection persisted through P90, though wave-1 amplitude in ovariectomized GluA3KO females remained intermediate between WT and sham KO groups. In contrast, P35 male GluA3KO mice showed no hearing deficits, and orchidectomy had no effect.

Conclusions: Our results show a potential effect of estradiol on AMPARs at inner hair cell-ribbon synapses only in females. In addition, we show that estradiol increases the vulnerability of the female GluA3KO mice to hidden hearing loss.

SU137. Novel Stella Platform Reveals Single-Molecule Dynamics of MYO7A-Driven Active Cargo Transport in Live Hair Cell Stereocilia

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Background: Unconventional myosins are motor proteins that function on the actin cytoskeleton. Genetic variants of four unconventional myosin genes, MYO3A, MYO6, MYO7A, and MYO15A, have been associated with human hereditary hearing loss. In particular, MYO7A is presumed to transport components of the mechanoelectrical transduction (MET) machinery as their “cargo” and anchor them to the actin core of stereocilia. However, the molecular mechanisms underlying myosin-driven active cargo transport in stereocilia are only partially understood. For example, it remains unclear whether MYO7A and other myosins function as a monomer like class-I myosins, a dimer like class-V myosins, or a higher-order oligomer like non-muscle class-II myosins. To fill this knowledge gap, we established a novel single-molecule imaging platform, STELLA (Single-Molecule Tracking and Enhanced Localization in Live Aural specimens), and investigated how MYO7A molecules traffic within stereocilia of live murine hair cells (Miyoshi et al., *Nature Communications*, 2025).

Methods: STELLA is currently built on a diSPIM light-sheet microscope (STELLA-diSPIM). Utricles and saccules were harvested from C57BL/6J mouse neonates at postnatal day 2–5 and cultured on a collagen matrix. MYO7A fragments fused with an N-terminal HaloTag were expressed in hair cells via Helios® gene gun transfection. HaloTag was fluorescently labeled with JFX554-ligands, which are brighter and more stable than fluorescent proteins, and visualized with a 561-nm laser light sheet. MYO7A motor activity was activated by several approaches, including chemical dimerization using the FKBP-AP20187 system, missense mutations destabilizing its autoinhibitory conformation, and tethering to the plasma membrane or actin filaments via the FKBP-FRB-Rapalog system.

Results: Under STELLA-diSPIM, MYO7A exhibited processive movement toward stereocilia tips when (1) chemically dimerized and (2) constitutively activated by missense mutations

disrupting its tail-mediated autoinhibitory mechanism. Velocity was 101 ± 53 nm/s ($n = 42$, mean $\pm$ SD) for dimers and 73 ± 47 nm/s ($n = 18$) for activated mutants, which were similar by Student's t-test. Wild-type MYO7A did not show directional motility at a detectable frequency. These results suggest that MYO7A traffics as a dimer within stereocilia when released from the autoinhibitory conformation. Cargo proteins of MYO7A, particularly SANS and harmonin (USH1C), may mediate this activation. MYO7A appears to exhibit stepwise motility when its tail is tethered to actin filaments via harmonin b fragments, which may represent how tip links adjust position and tension in mature stereocilia.

Conclusions: Through STELLA microscopy, we established real-time dynamics analysis of protein molecules within live hair cells. Such methods will remain essential tools in hearing research, as the intracellular environment of inner ear hair cells is not easily reconstituted. STELLA can also be used to functionally evaluate and reclassify genetic variants of uncertain significance (VUS), particularly to identify those with hidden pathogenicity that may mislead clinical decision-making.

SU138. FOXO3 Protects Hearing in Mice by Maintaining Outer Hair Cell Mitochondrial Function

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Background: Noise induced hearing loss (NIHL) affects around 40 million adults in the United States, but little is known about genetic susceptibility to NIHL. FOXO3 is a conserved transcription factor with some genetic variants that are strongly linked to human longevity. We have previously found that homozygous Foxo3 knockout mice (Foxo3-KO) are more susceptible to noise damage than their wild-type littermates. Among other issues, outer hair cells (OHCs) in Foxo3-KO mice appear primed to die in response to a mild noise exposure. Here we compare the transcriptome of undamaged OHCs from wild-type and Foxo3-KO adult mice, in order to identify some of the mechanisms for this damage susceptibility.

Methods: A mouse line was created that harbored both the inducible CRE recombinase under control of the Slc26a5 (Prestin) promoter and the HA-tagged RPL22 (Ribotag) protein, which is incorporated into translating polyribosomes. These mice were bred to the Foxo3-KO line to obtain mice with one allele of Slc26a5-iCRE, one or two alleles of RPL22(HA), and either two wild-type or two knockout alleles of Foxo3. Mice were injected with tamoxifen to enable expression of RPL22(HA) in outer hair cells, which was validated by immunostaining. Immunoprecipitation was used to enrich for HA+ ribosomes from both wild-type and Foxo3-KO tissues. RNA samples were reverse-transcribed into cDNA and sequenced. Sequencing validation was performed with qPCR. Gene ontology terms were identified using R Studio. Adult OHC mitochondria were assessed using immunofluorescence, confocal microscopy, and blinded analysis in Imaris. OHCs in live preparations of organ of Corti from both genotypes were visualized with MitoTracker Green. Mitochondrial membrane potential was assessed with tetramethylrhodamine, calcium levels with rhodamine-2 AM, and superoxide levels with MitoSox.

Results: OHC expression of HA-tagged RPL22 was validated in in tamoxifen-injected littermates. Selected differentially expressed genes (DEGs) were validated by qPCR. Gene ontology analysis suggested that many terms associated with energy production differed between the genotypes. In particular, wild-type mice expressed higher levels of genes associated with mitochondrial maintenance, including the FOXO3 target Pink1. Morphologically, individual adult Foxo3-KO OHCs displayed equal volumes of mitochondria overall compared to OHCs in wild-types. However, individual mitochondria within Foxo3-KO OHCs were significantly larger, suggesting changes in mitochondrial dynamics. The live OHC mitochondrial analyses are ongoing, and results will be presented.

Conclusions: Mitochondrial dysfunction has previously been associated with hearing loss from noise, with some reports indicating that it may also play a role in NIHL. These findings suggest that FOXO3 promotes OHC survival after noise exposure in part through maintaining mitochondrial integrity and/or function. Understanding how mitochondria are involved in OHC resilience could lead to future interventions that help protect hearing from noise damage.

SU139. The Role of O-GlcNAcylation in the Inner Ear

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Background: Post-translational modifications are essential for fine-tuning protein function by altering their activity, localization, and interactions with other molecules. O-GlcNAcylation (O-GlcNAc) is a posttranslational modification present across various tissues. This modification is dynamically regulated by only two key enzymes: O-GlcNAc transferase (OGT), which adds the N-acetylglucosamine moiety, and O-GlcNAcase (OGA), which removes it, making it an attractive target for pharmacological manipulation. However, its role in inner ear physiology remains largely unexplored.

Methods: Here we investigated the role of O-GlcNAcylation in the inner ear, using electrophysiological patch-clamp measurements and immunohistochemical stainings in inner hair cells and spiral ganglion neurons in acutely isolated cochlear tissue from mice.

Results: In this study, we demonstrate for the first time the presence of O-GlcNAcylation in multiple inner ear cell types, including auditory sensory epithelial cells. Pharmacological modulation of OGT and OGA activity alters O-GlcNAcylation levels in these cells, indicating that this modification is active and regulated in the inner ear. Moreover, patch-clamp recording after the inhibition of OGT and OGA reveals alterations in voltage-gated K⁺, and Ca²⁺ channels, in inner hair cells and spiral ganglion neurons.

Conclusions: These findings suggest a potential regulatory role for O-GlcNAcylation in inner ear function and open new avenues for investigating its contribution to auditory physiology and pathology.

SU140. Further Characterization (Structural, Quantitative and Multi-Omic) of Inner Ear Mitochondria

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Background: In this study, we quantified multiple aspects of mitochondrial structure in the vestibular inner ear. Our main hypothesis in this research is that mitochondria are structurally heterogeneous, depending on which hair cell (HC) type is under consideration and the specific locations of mitochondria in relation to other hair cell organelles. One such structural relationship that we have reported on previously is the polarization of mitochondrial crista junctions (CJs), openings of the internal mitochondrial membrane into the intermembrane space that is likely responsible for transport of molecules from the mitochondrial matrix out to the cytoplasm.

Methods: Inner ear vestibular tissue obtained from Long-Evans rats under an approved UIC IACUC procedure was used for electron microscope tomography. Data were processed with the IMOD (v.3.13.6) software package (Kremer et al., 1996) and serial tomograms were joined using the etomo subroutine (IMOD). Manual segmentation was done with the 3dmod subroutine (IMOD) to obtain final models. Reconstructions were visualized using 3dmod (IMOD), including its SLICER option, to track relevant features in 3 dimensions. Several IMOD drawing tools (Sculpt, Join, Warp, Interpolator) and a proximity analysis tool (Mtk) with a randomization feature, were also used. Proteomic and genomic analyses are also being performed on the 3 different size classes.

Results: Quantitative analyses were performed to test our hypothesis, including total mitochondrial counts in both types of vestibular HCs (compared to cochlear HCs), efferent boutons, and afferent calyces, counts of CJs on either side of a mitochondrion relative to other cellular organelles, analyzing polarization ratios vs distances, bioenergetic calculations of ATP production, and an Mtk proximity analysis test, using CJ randomization as a control. Results support our hypothesis of structural heterogeneity of mitochondria, as CJs are polarized toward specific organelles within the cell and appear to be non-randomly distributed. Finally, vestibular HCs have < half the number of mitochondria compared to cochlear HCs, although some are larger than cochlear mitochondria.

Conclusions: These results have functional implications for the various structural differences. CJ polarization toward organelles that use energy can transport ATP more efficiently. For example, the cuticular plate is an organelle that functions to return stereocilia rootlets back to their original position. Our results show a non-random orientation of mitochondrial crista junctions toward the cuticular plate. These structural differences may provide key information to better understand their function and to address mitochondrial deafness and dizziness disorders.

SU141. MYO3A and MYO3B Regulate Stereocilia Tip Widening via Their Kinase and Motor Domains

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Background: The formation of stereocilia depends on tightly regulated actin dynamics and the targeted delivery of actin modulating proteins by non muscle myosins. Class III myosins, MYO3A and MYO3B, localize the actin binding proteins ESPN1 and ESPNL to stereocilia tips and contribute to stereocilia widening. Although mutations in Myo3a cause several hereditary hearing disorders, including DFNB30, the underlying pathogenic mechanisms remain poorly

understood. Both MYO3A and MYO3B possess an N terminal kinase domain that phosphorylates the motor region, thereby attenuating motor activity. In this study we dissect the distinct and overlapping contributions of MYO3A and MYO3B to hair bundle development.

Methods: We generated a series of mouse models: a Myo3a knockout, a Myo3a / Espn1 double knockout (to test whether MYO3B requires ESPN1 for tip localization), and a Myo3a knockout combined with a Myo3b allele bearing a four amino acid deletion in the N terminal kinase domain. Protein localization and hair bundle architecture were examined using high resolution Airyscan microscopy and expansion microscopy.

Results: Loss of MYO3A produced markedly elongated stereocilia throughout the cochlea, establishing its role in limiting stereociliary elongation. Although MYO3B tip localization increased in the absence of MYO3A, it offered only modest compensation; MYO3B failed to control short actin filament levels at stereocilia tips or to transport ESPN1 effectively. Strikingly, simultaneous deletion of MYO3A and ESPN1 yielded a distinct phenotype characterized by the loss of MYO3B from stereocilia tips and a sharp tapering of the tip region, indicating that MYO3 complexes specifically modulate actin incorporation along the upper shaft. Moreover, in the absence of MYO3A, the kinase deficient MYO3B mutant increased stereocilia length and altered distribution of MYO7A and EPS8L2, revealing an unexpected role for the kinase domain in regulating tip localized actin dynamics.

Conclusions: Our findings establish MYO3A as the dominant class III myosin in inner hair cells, essential for preserving normal stereociliary dimensions. The data illuminate how MYO3A mediated actin regulation shapes hair bundles and suggest mechanistic pathways by which MYO3A dysfunction contributes to DFNB30 associated deafness.

SU142. Functions of CCDC181 in Kinocilium Formation and Auditory Perception

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Background: Auditory hair cells exhibit a highly specialized ciliary architecture, consisting of a bundle of stereocilia that is responsible for the mechanotransduction and a single kinocilium that governs hair bundle polarity during development. In murine cochlear hair cells, the kinocilium appears during embryonic development alongside the formation of stereocilia bundles, and it degenerates by around postnatal day 12 as the stereocilia mature. Despite this well-defined developmental timeline, the molecular mechanisms governing kinocilium formation and subsequent degeneration remain largely unknown.

Methods: immunohistochemical staining, ABR, SEM, knockout mouse

Results: Through immunohistochemical staining, we found that CCDC181, a microtubule-binding protein, is abundantly expressed in the postnatal cochlear hair cells and specifically localized to the kinocilium. To investigate its function in kinocilium development, we generated a Ccdc181 knockout mouse. Hair cell morphology was characterized by using immunostaining and electron microscopy, and auditory function was then evaluated by measuring auditory brainstem responses.

Conclusions: Our preliminary data suggest that ablation of CCDC181 has minimal effects on kinocilium formation, hair cell polarity, or auditory function in mice.

SU143. Characterizing the Mechanosensitive Recruitment and Protein Partners of Hair Cell Repair Protein XIRP2

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Background: Hearing depends on the precise function of auditory hair cells, whose actin-based stereocilia deflect in response to sound to activate mechanotransduction channels. Hair cell death and damage to hair cell structures, such as stereocilia, can contribute to hearing loss. Therefore, understanding endogenous repair mechanisms is crucial for developing therapeutic targets for progressive hearing loss. Our lab previously found that noise exposure induces F-actin damage in stereocilia that is subsequently repaired, suggesting an endogenous repair process. A critical question remains: how do hair cells sense and repair F-actin damage, and how does this process prevent hearing loss? XIRP2, an actin-associated protein enriched in stereocilia, accumulates at damage sites, and Xirp2 knockout mice show impaired hair cell repair and progressive hearing loss. I hypothesize that XIRP2 is a key player in the hair cell actin repair process, working in concert with other stereocilia proteins that we aim to identify using proteomic approaches.

Methods: To investigate XIRP2's role in actin repair, we used two complementary approaches. First, we employed an in vitro model of actin damage to identify which domain of XIRP2 mediates its recruitment to F-actin lesions. We used laser photoablation to induce localized strain sites in stress fibers of fibroblast cells transfected with full-length or truncated XIRP2 constructs. Recruitment indices of each construct were compared to assess mechanosensitivity.

Second, to identify other proteins involved in hair cell repair, we developed a proximity labeling strategy in vivo. We generated a TurboID-XIRP2 knock-in mouse model, enabling biotinylation of proteins near XIRP2 in the context of F-actin damage. These proteins were then isolated and analyzed by mass spectrometry to define potential XIRP2 interactors.

Results: Our in vitro experiments indicate that XIRP2 is recruited to damaged actin in a force-dependent manner, and we have mapped the minimal mechanosensitive domain responsible for this recruitment. We also verified normal expression and localization of XIRP2 in the TurboID knock-in model. Mass spectrometry analyses identified candidate interactors that may participate in actin repair.

Conclusions: XIRP2's recruitment to actin damage requires its minimal mechanosensitive domain. Using our TurboID-XIRP2 knock-in strategy, we created and validated a new proximity labeling approach in auditory hair cells. This platform not only revealed candidate proteins involved in stereocilia repair but also establishes a broadly applicable method for studying protein interactions in the inner ear. By expanding the toolkit for proteomics in hair cells, this work provides new opportunities to uncover pathways that could be involved in progressive hearing loss.

SU144. Mechanoelectrical Transduction in Cnidarians Nematocytes and the Evolutionary Origin of Vertebrate Hair Cells

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Background: The conversion of mechanical stimuli to electrical signals is of fundamental importance in many physiological responses, including auditory and vestibular functions in vertebrates, and a host of sensory functions in invertebrates. Vertebrate inner ear hair cells contain a mechanoelectrical transduction (MET) complex located at the tips of stereocilia. Transmembrane Channel-like 1 and 2 (TMC-1/2) proteins in complex with have been suggested as the key mechanosensitive channel in this complex. Biochemical and structural characterization of the MET complex in vertebrate hair cells has been, and continues to be a major challenge.

Methods: We pursued morphological pharmacological, functional, bioinformatic and reverse genetic approaches to evaluate the evolutionary relationship between the cnidarian nematocyte and vertebrate hair cells.

Results: Here, we present new evidence pointing to a relationship between the vertebrate hair cell and the Cnidarian stereocilia-containing hair cells, which include nematocytes and other sensory ectodermal cells. Single cell differential transcriptomics together with targeted knock down of candidate mechanosensitive molecules, directly point to members of the TMC family of ion channels/scramblases as key participants in the generation of mechanically-evoked receptor potentials and the eversion of cnidocysts. Furthermore, dependence on extracellular Ca²⁺, pharmacological susceptibility to ototoxic aminoglycoside antibiotics and the spontaneous accumulation of the water-soluble styrene fluorophore FM1-43 also support an evolutionary relation between a putative MET complex in nematocytes and its equivalent in vertebrate hair cells.

Conclusions: Our computational, biochemical, and genetic studies corroborate a direct link to vertebrate hair cells and point to evolutionarily conserved mechanotransduction mechanisms.

SU145. The Role of PCDH15-CD3 in Stability of Tip Links in Mammalian Auditory Hair Cells

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Background: Sound detection is initiated by deflection of stereocilia, mechanosensitive projections at the apex of auditory hair cells. Stereocilia are arranged in rows of increasing heights and interconnected by extracellular tip links that convey forces to the mechano-electrical transduction (MET) channels. Tip links are formed by cadherin-23 (CDH23) and protocadherin-15 (PCDH15), at the upper and lower ends of a tip link, respectively. Mutations in genes encoding these proteins result in deafness. Hair cells express only one isoform of CDH23 but multiple isoforms of PCDH15, grouped into three classes (CD1, CD2, and CD3). Though both PCDH15-CD3 and PCDH15-CD2 are localized at the tips of stereocilia, only PCDH15-CD3 is

localized exclusively at the tips of transducing 2nd and 3rd row stereocilia within a hair bundle, suggesting the PCDH15-CD3 is the isoform class forming tips links. Yet, *Pcdh15-CD3*^{-/-} mice have normal hearing thresholds and overexpress PCDH15-CD2, indicating potential compensation by CD2. Further, disruption of tip links with Ca²⁺-free extracellular media results in the disappearance of PCDH15-CD3 immunolabeling, while PCDH15-CD2 immunolabeling is still present. We hypothesize that tip links of *Pcdh15-CD3*^{-/-} mice may have different stability and recovery after disruption.

Methods: We generated a novel mouse strain lacking only CD3 isoform of *Pcdh15* (*Pcdh15-CD3*^{-/-}). To study the role of CD3 isoform in tip link breakage and regeneration, we incubated organ of Corti explants (from *Pcdh15-CD3*^{-/-}, *Pcdh15-CD3*^{+/-}, and *Pcdh15-CD3*^{+/+} mice) in Ca²⁺-free medium (supplemented with 5 mM of BAPTA) for one minute to break the tip links and then followed tip link recovery in regular Ca²⁺-containing media at 0, 3, 6, 9, and 12 hours after breakage using high-resolution scanning electron microscopy (SEM). Control samples were incubated in regular Ca²⁺-containing HBSS (1.26 mM of Ca²⁺) and the tip links were visualized by SEM and counted at the same time points after incubation.

Results: 1) Shorter incubation in Ca²⁺-free medium supplemented with BAPTA (one minute instead of 15 minutes used previously) results in effective disruption of at least 80% of the tip links in wildtype inner hair cells but followed by a faster tip link recovery. 2) Tip links in *Pcdh15-CD3*^{-/-} hair cells recover more slowly after disruption. 3) Most strikingly, more tip links (up to 40%) are present in *Pcdh15-CD3*^{-/-} inner hair cells immediately after disruption with BAPTA and many of them appear to be shorter than normal.

Conclusions: So far, our data argues that the CD3 isoform is compensated by PCDH15-CD2 in *Pcdh15-CD3*^{-/-} hair cells, and these tip links are more resilient to disruption. The shorter length of these tip links also indicates that temporary homomeric PCDH15-PCDH15 links could be present due to overexpression of PCDH15-CD2.

SU146. Persistent Remodeling of Hair Cell Mitochondrial Networks in Response to Low Dose Cisplatin Exposure

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Background: Cisplatin is a widely used chemotherapeutic agent with broad efficacy. However, its clinical utility is limited by dose-dependent toxicities, including ototoxicity that can lead to permanent hearing loss.

While high-dose cisplatin-induced hair cell apoptosis has been well characterized, the cellular effects of clinically relevant, sub-lethal doses remain poorly understood. Notably, some patients experience delayed-onset, progressive hearing loss after treatment, suggesting that surviving hair cells may undergo lasting subcellular stress. Mitochondria have been identified as early and critical targets of cisplatin-induced death in hair cells (Lee, 2024), yet how mitochondrial structure and function respond to low dose, non-lethal exposure has not been fully explored.

In this study, we used a zebrafish model to investigate the effects of low dose cisplatin exposure on hair cell mitochondria. We assessed mitochondrial morphology, calcium dynamics, and membrane potential as indicators of early dysfunction, to better understand low dose cisplatin-induced hair cell injury and identify potential mechanisms of vulnerability and repair.

Methods: Transgenic zebrafish larvae expressing a mitochondrial calcium indicator in hair cells (Tg(myo6b:mitoGCaMP)) were exposed to (30 μ M, 100 μ M, 300 μ M, 1 mM) cisplatin for 2 hours, followed by (0, 6, 24, 48, 72) hour recovery. Larvae were then fixed in paraformaldehyde and imaged using super-resolution confocal microscopy. Mitochondrial morphology was quantified using 3D image analysis. Ongoing studies include live imaging of mitochondrial membrane potential with TMRE and time-lapse super-resolution microscopy to assess dynamic mitochondrial remodeling. Functional consequences are being evaluated using a microflume-based rheotaxis assay to detect subtle deficits in hair cell-mediated behavior.

Results: Low dose cisplatin exposure induced significant changes in mitochondrial morphology in neuromast hair cells, even in the absence of overt hair cell loss. Our data revealed time and dose dependent changes in morphology features (count, branch length, branch junctions, sphericity) associated with mitochondrial network complexity. Notably, we observed the formation of toroidal (“donut”) mitochondria, which increased in frequency with higher cisplatin doses and persisted through the recovery period. Our data also suggests mitochondrial calcium dysregulation and loss of mitochondrial membrane potential. These changes were dose-dependent and evolved throughout the recovery period, with distinct morphological and functional trajectories observed at different concentrations.

Conclusions: Our findings show even clinically relevant, low dose, cisplatin causes persistent disruption of hair cell mitochondrial morphology and functionality. The persistence of mitochondrial morphological abnormalities supports a model in which hair cells experience chronic subcellular stress, potentially priming them for later vulnerability to additional insults, such as noise or aging. These insights lay the groundwork for developing targeted strategies to preserve hearing in patients undergoing cisplatin chemotherapy.

SU147. Live Imaging of Presynaptic Ribbons in the Aging Mouse Cochlea

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Background: Ribbon synapses in inner hair cells (IHCs) mediate rapid and precise neurotransmission to spiral ganglion neurons (SGNs), enabling accurate auditory signaling. With advancing age, both humans and mice experience synaptic alterations that contribute to hearing impairment. However, the cellular mechanisms underlying age-related synaptic decline remain poorly defined. Direct observation of presynaptic ribbon behavior across the lifespan may clarify how synaptic architecture changes with aging.

Methods: We used RIBEYE-tagRFP mice to visualize presynaptic ribbons in cochlear explants at three age groups: 3–4 months, 6 months, and 17–18 months. Whole-mount preparations were imaged with a Nikon A1R confocal microscope equipped with a 60 $\times$ water immersion objective. Confocal z-stacks spanning the IHC region were acquired, and RIBEYE puncta were analyzed using Imaris for number, size, and motility parameters. Group comparisons were made across age cohorts to identify trends in ribbon morphology and dynamics.

Results: Age was associated with progressive changes in presynaptic ribbon architecture. The total number of RIBEYE puncta decreased from young adult (3–4 months) to middle-aged (6 months) and further into aged (17–18 months) cochleae. In parallel, the average puncta size increased with age, suggesting enlargement or aggregation of remaining ribbons. Despite these morphological changes, ribbon motility, as assessed by speed of movement, remained similar across all three age groups. These findings indicate that while the structural landscape of synapses is altered during aging, the intrinsic dynamic properties of ribbon mobility are preserved.

Conclusions: This study demonstrates an age-dependent decline in ribbon number accompanied by enlargement of surviving puncta, consistent with synaptic remodeling in the aging cochlea. The preserved motility of ribbons across ages suggests that dynamic capacity for movement is maintained, even as synapse density diminishes. These data support a model in which aging preferentially affects ribbon abundance and morphology, rather than mobility. Ongoing work incorporating auditory brainstem response (ABR) measurements will link these synaptic changes to hearing function. Together, these findings highlight presynaptic ribbon remodeling as a potential contributor to age-related auditory decline and establish live imaging of RIBEYE-tagRFP cochleae as a powerful tool for studying mechanisms of synaptic aging.

SU148. Rapid Peri/Post-Mortem Technique for Harvesting and Dissecting Human Temporal Bones That Circumvents Extended Decalcification

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Background: The otic capsule, the densest bone in the body, encases the inner ear structures the cochlea, vestibule, and semicircular canals. Postmortem temporal bone processing typically requires slow fixation and prolonged decalcification taking weeks to months, which can compromise molecular integrity. Here, we present a novel method for rapid fixation and whole-mount dissection of the human cochlea and vestibular end-organs harvested peri/postmortem from human organ donors. This approach minimizes decalcification time, preserves the tissue's structural integrity, facilitates rapid histological analysis, and enables future proteomic and multi-omic studies.

Methods: We have developed an efficient workflow, in collaboration with the federally designated organ recovery organization for Maryland, Virginia, and Washington, DC to harvest temporal bones from organ donors within a very short peri/post-mortem interval of 1 to 2 hours. Using an extracranial approach with a bone plug autopsy saw, specimens were collected and fixed in 10% formalin after disarticulating the stapes footplate. The preparation was subsequently trimmed using a surgical drill in two stages: first, the bone plug was reduced to the otic capsule; second, the various neurosensory tissues were collected, beginning with the vestibular organs (the three cristae, utricle, and saccule) and concluding with delicate drilling around the cochlear capsule, followed by dislocation of the cochlear sensory epithelium from the modiolus. A brief 24-hour decalcification in 14% EDTA was then performed to decalcify the spiral laminae and otoconia.

Results: Whole-mount cochlea with minimal mechanical damage was obtained, as well as round window membrane, ossicles, and spiral ganglion neurons from the Rosenthal canal. In addition, all five vestibular organs—the lateral, anterior, and posterior cristae, the utricle, and the saccule—were harvested. These structures were imaged under light microscopy generating a unique image library of human inner ear structures and nerve fibers. The resulting tissue is suitable for histology, immunostaining, and multi-omics analysis.

Conclusions: This method enables successful harvest of intact cochlear and vestibular organs within hours of death, eliminating the need for extended decalcification, and preserving tissues for histology and multi-omic analyses. This represents a significant advance for human inner ear research.

SU149. Anatomical Integrity of the Human Organ of Corti Visualized Using Paired Optical Coherence Tomography and Histology

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Background: Human cochlear microstructures are delicate and hard to access, making it difficult to noninvasively image intracochlear anatomy and pathology. Towards this need, optical coherence tomography (OCT) has been proposed to visualize OoC structures in situ in fresh human cochleae through the intact round window membrane (RWM). However, granular OCT images cannot reliably identify the distinct cell types that make up the OoC. To more confidently interpret OCT images, we analyzed histology obtained from the same donor for direct comparison between OCT features and histologic structure.

Methods: Pairs of fresh temporal bones (4-24 hours postmortem) were obtained with IRB approval from the Mass General Hospital from donors without a history of unilateral ear disease. One ear was prepared for OCT imaging and the contralateral ear was immediately fixed. After the middle ear cavity was surgically opened to access the cochlea, OCT images were collected from unfixed cochleae through the intact RWM using a 900-nm center wavelength OCT system (Gan6201C1, Thorlabs, Germany). Time of imaging was 10-28 hours postmortem. Following imaging, both ears were histologically processed using a rapid, non-decalcifying histological processing workflow developed in our lab: whole bony specimens were fluorescently labeled and embedded in resin (Technovit 9100NEW®). Embedded blocks were oriented to align the histological sectioning plane with the OCT imaging plane using a customized disc sander. Thin (~100 µm) sections were produced with precision diamond wire saws, followed by confocal and super-resolution microscopy to visualize cochlear microstructures. OCT–histology registration was performed at matched cochlear locations, with 3D reconstructions used to confirm correspondence.

Results: OCT images and histological sections from the same cochlear location across paired human temporal bones were obtained. There was high contralateral symmetry in cochlear morphology within individuals. Direct registration enabled confident interpretation of granular OCT features; e.g., differentiating intact versus collapsed OoC architecture and recognizing

regions indicative of cell loss, all based on histologic ground truth. These comparisons establish OCT signatures for the human OoC that correspond to graded microanatomical pathology.

Conclusions: Paired OCT images and histology registered from the same donor showed similar OoC anatomy and verified our ability to accurately interpret OCT images. OoC anatomy across individuals varied, and pathological states could be differentiated. These results demonstrate the capability of OCT to non-traumatically visualize cochlear integrity through the RWM, highlighting its potential as a diagnostic tool. The ability to determine the presence of hair cells and supporting cells in live patients would enable more precise individualized assessment with potential to guide emerging therapies (e.g., gene- and regenerative approaches). In vivo OCT could assist in targeted therapies, determining anatomic barriers to local delivery such as false round-window membrane, and prevent trauma such as fractures of the osseous spiral lamina.

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SU150. The Inner Ear in NF2-Related Schwannomatosis: Histological and Molecular Perspectives

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Background: NF2-SWN is a rare autosomal dominant genetic disorder characterised by bilateral vestibular schwannomas and progressive hearing loss. It results from pathogenic variants in the NF2 gene, located on chromosome 22q, which lead to loss or dysfunction of the tumour suppressor protein Merlin. Although vestibular schwannomas commonly occur as sporadic, unilateral tumours, NF2-SWN is distinguished by its inherited nature and earlier onset. While tumour compression of neural structures is well recognised, mechanisms of sensory loss beyond tumour burden remain unclear. We are currently studying archival human temporal bones (Brown et al., Hearing Research, 2025) and a mouse model to investigate inner ear pathologies in NF2-SWN.

Methods: Human temporal bone sections stained with haematoxylin and eosin were assessed for cochlear and vestibular degeneration using light microscopy (subject ages 14-65; samples held under UK Human Tissue Authority licence #12161). A conditional knockout mouse model (Postn-Cre; Nf2flox/flox; Gelhausen et al., J Neurosci, 2015) was used to examine inner ear pathology via immunofluorescence. Antibodies targeting endogenous total merlin (DS3SW, Cell Signaling Technology), Aldh1a1 (tumour marker), and Iba1 (macrophage marker) were used to identify molecular and cellular changes. Cre-negative littermates served as controls.

Results: Human samples with bilateral vestibular schwannomas showed widespread degeneration of cochlear and vestibular structures, including loss of spiral ganglion neurons and nerve fibres, disorganisation of the organ of Corti, and Schwann cell proliferation with subsequent nerve compression. Some tumours had a complex composition, with evidence of mixed schwannomas and meningiomas. In 3-month-old Postn-Cre; Nf2flox/flox mice, increased localised expression of Aldh1a1 (but without evidence of tumour growth) and increased numbers of Iba1-labelled macrophages within neural tissues possibly indicated early inflammatory and metabolic stress responses. These changes were absent in Cre-negative controls.

Conclusions: These findings suggest that hearing loss in NF2-SWN may begin with subtle, tumour-independent changes within the cochlea and auditory nerve, linking NF2 gene loss to early sensory dysfunction. Elevated Aldh1a1 expression and increased macrophage indicate that inflammatory and structural mechanisms are active prior to tumour progression. Together, these processes likely contribute to hearing loss and other symptoms. A multi-layered therapeutic approach – targeting both early, non-tumour related changes and later tumour-driven effects – may more effectively preserve hearing and improve outcomes in NF2-SWN.

SU151. Loss of Orexin 2 Receptor Signaling Exacerbates Noise-Induced Hearing Loss

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Background: Orexins, also known as hypocretins, are neuropeptides produced by hypothalamic neurons involved in the sleep-wake cycle, food intake, and autonomic output. We discovered that orexin ligands and receptors are also expressed in the cochlea. Mutation of orexin 2 receptor (Ox2R) leads to early onset narcolepsy. Dual orexin receptor antagonist (DORA) medications are used to treat insomnia with new drugs targeting orexin signaling being tested. Given the previously unrecognized expression of orexin signaling molecules within the cochlea, constitutive loss or knock down of orexin through prolonged DORA medications could alter mechanisms underlying normal hearing sensitivity and/or resilience against damage. We begin to address this by examining hearing in constitutive Ox2R mouse mutants subjected to noise.

Methods: Ox2R wild-type (WT) and knockout (KO) mice were used to investigate the role of Ox2R in moderate noise-induced hearing loss. We used Auditory Brainstem Response (ABR) testing and anatomical assessments to analyze effects of noise on physiological and morphological integrity of the cochleae of Ox2R WT and KO mice. Half of the mice (WT and KO groups) were noise exposed (97dB SPL, 8-16kHz, 2h). ABRs were performed before noise exposure to measure baseline hearing thresholds and post-noise periodically through 21 days to track hearing threshold recovery on all mice. A second cohort of WT and KO mice, under identical conditions as the first, underwent transcardial perfusion (4% paraformaldehyde) 5 days post-noise exposure. The temporal bones of WT and KO mice were isolated, processed, cryostat-sectioned, and hematoxylin and eosin (H and E) stained. Immunohistochemistry was performed on alternate sections using primary antibodies (Ox1R, Aqp1, F4/80) and appropriate secondary antibodies. Photomicroscopy was performed (Leitz DSM and Zeiss LSM880 Confocal).

Results: Hearing thresholds of Ox2R WT noise-exposed mice recovered after 5 days post-noise while threshold shifts in Ox2R KO noise-exposed mice remained elevated and began to decline at 7 days post-noise. Ox2R WT thresholds returned close to baseline while Ox2R KO thresholds did not decline to similar levels by day 21 post noise, indicating that a noise-induced temporary threshold shift in WTs became a permanent shift in KOs. In noise-exposed KO mice, there were morphological changes (H and E) in the stria vascularis, decreased protein localization of i) Ox1R in supporting cells and ii) aquaporin 1 in the spiral ligament, and increased F4/80 localization in the spiral ligament.

Conclusions: The functional and anatomical hearing-related changes observed in our mouse model of constitutive Ox2R loss-of-function may reflect constitutive gene loss in human

narcolepsy and could mirror effects following potential long-term use of DORA medications. The effects on hearing may be predictive of patients who are using drugs long-term which alter the orexin signaling system, potentially putting them at risk for augmented hearing loss.

SU152. The Auditory-Nerve Initial-Peak (ANIP) Response to High-Level Clicks is From a Unidirectional Stereocilia Deflection Away From the Nearest Saturation of OHC Mechano-Electric Transduction

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Background: The auditory-nerve initial peak (ANIP) response is a mysterious response that doesn't fit with most conceptions of how the cochlea works. It is the earliest response from moderate-to-high-level rarefaction clicks in cat auditory-nerve (AN), type-1 fibers with characteristic frequencies (CFs) < 3 kHz. It was first shown to be unusual by its strong inhibition by stimulation of medial-olivocochlear (MOC) efferents, which contrasted with the lack of MOC inhibition of the first peak of basilar membrane click responses. The MOC inhibition of ANIP indicates that the ANIP response is affected, or generated, by outer-hair-cell (OHC) motility. Consistent with this, the ANIP response was shown to be suppressed when high-level, low-frequency "bias" tones quasi-statically pushed the OHC mechano-electric transduction (MET) function into low-slope, saturating edge regions. Contrary to prior theory, our preliminary results showed that the bias-tone phase that produced the most suppression (the major-suppression phase) was opposite for ANIP responses compared to low-level click or tone responses.

Methods: In anesthetized cats, we recorded single-fiber AN responses, in response to low-level clicks, low-level tones, and ANIP-producing, higher-level clicks, with/without 50 Hz bias tones. We used new methods to exclude the AN-fiber excitation from high-level bias-tones, so that this excitation doesn't obscure bias-tone suppression of AN responses.

Results: The major suppression phases for ANIP responses were nearly opposite (~180 degrees different), compared to those of low-level clicks and tones.

Conclusions: An "ANIP-MET-asymmetry hypothesis" is consistent with the results: Low-level responses take several cycles to build up and depend on the local slope in both directions of the MET function; their major suppression phase is when the bias tone quasi-statically deflects the MET operating point to its nearest low-slope, saturation edge. In contrast, while ANIP responses oscillate for many cycles like low-level responses, ANIP is only the first half cycle and requires an OHC stereocilia deflection only in one direction. When a bias tone puts the OHC-MET operating point at its nearest saturating edge, low-level, multi-cycle responses are suppressed, but a rarefaction-click's unidirectional initial stereocilia deflection is away from this saturation, enabling a large traverse of the MET function and a large depolarizing OHC current. The ANIP major suppression arises from bias-tone deflections to the MET-function opposite saturation. Our presentation will point out that the ANIP-MET-asymmetry hypothesis fits well with the new theory that cochlear amplification of low-level responses is due to OHC motility changing the local OoC cross-sectional area at a phase that adds energy to the traveling wave in the scala media-tympani fluid. The ANIP-MET-asymmetry hypothesis also fits with low-level responses

being carried primarily by the traveling wave while ANIP is carried by a cortilymph wave, with both waves accumulating gain as they travel to the CF region.

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SU153. Investigation on Organ of Corti Micro-Mechanics and Inner Hair Cell Stereocilia Stimulation Mechanisms Through 3D Finite Element Models of the Mouse Organ of Corti

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Background: The intricate cytoarchitecture of the organ of Corti (OoC) stimulates inner hair cell (IHC) stereocilia, triggering mechano-electrical transduction (MET) channels. Recent advancements in Optical Coherent Tomography (OCT) technology allow detailed motion measurement of the tectorial membrane (TM) and reticular lamina (RL) relative to the motion of basilar membrane (BM) in both transverse and radial directions. High speed camera imaging also revealed stereocilia motion relative to RL radial direction motion. These experimental data enable modeling effort to capture and explain OoC micro-mechanics, but most modeling effort still lack such details. A slice modeling technique using a 3D Finite Element Model (FEM) for a cochlea with Floquet boundary conditions meets the required details with reasonable computational cost (Wang and Puria, 2025, HR).

Methods: A 3D Finite Element Model (FEM) of a slice of the mouse OoC of the apex was built. The cellular structure of the OoC is reconstructed based on 2-photon measurements on fresh tissue (Soons et al. 2015), as well as Scanning Electron Microscopy and histology slices on fixed tissues (Davies and Forge 1987, Taylor et al. 2012). The model captures the realistic representation of the Deiter cells, Outer Hair Cells (OHCs), IHCs, inner and outer pillar cells, Hensen cells, as well as the fluid space of the inner sulcus, tectorial membrane, and the cortilymph consisting of the inner tunnel of Corti, the space of Nuel, and the outer tunnel. It includes the longitudinal inclination of the outer hair cells, Deiter cells, phalangeal processes and the outer pillar cells resulting in the mosaic pattern of the reticular lamina. The material properties were determined using Machine Learning algorithm to fit experiment data on mouse apex (Lee et al. 2016).

Results: The slice model was able to fit the experimental data of the passive postmortem measurements with reasonable tolerance. The TM and RL motion relative to BM motion were observed. Besides being able to fit the data, we are also able to identify the contribution of the stiffness of each structure, such as TM and RL, on the micro-mechanical motion of the OoC. For example, the stiffness of the OHCs and pillar cells have more significant effect on the motion of the RL than RL stiffness itself. The relationship of the stereocilia motion and its surrounding fluid motion are observed. The two key hypotheses that the model tests are: 1) whether the IHC stereocilia are stimulated by inner sulcus fluid, and 2) the effect of stimulation mode on frequency.

Conclusions: FEM slice cochlear model is a powerful tool to study cochlear OoC micro-mechanics and test IHC stereocilia stimulation mechanism hypotheses. Work supported by R01 DC007910 and R21 DC022922 from the NIDCD of NIH.

SU154. Outer Tunnel Vibration Within the Cochlear Partition of the Basal Turn in Sensitive Gerbil Cochleae

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Background: The outer tunnel is the largest fluid-filled space within the auditory sensory organ, the organ of Corti, in the basal turn of the gerbil cochlea. It consists of motile outer hair cells, the soma and the phalangeal processes of the Deiters' cells. The phalangeal processes connect the somatic cupula of the Deiters' cells to the cuticular plate of the outer hair cells, forming part of the reticular lamina. Despite its structural prominence, the functional role of the outer tunnel in cochlear mechanics remains poorly understood.

Methods: Young Mongolian gerbils of either sex with normal hearing were used in this experiment. The bulla on the left side was opened through a ventrolateral surgical approach. After the stapedial artery was removed from the bony surface of the cochlea, an opening was made in the lateral wall of the scala tympani of the basal turn. When the cochlear partition was positioned approximately in the horizontal plane, the magnitude and phase patterns of the vibrations were measured at the best frequency and frequencies more than one octave below the best frequency from the cross-sections of the outer tunnel and the cochlear partition using a scanning heterodyne low-coherence interferometer.

Results: At the best frequency, all structures of the outer tunnel and the cochlear partition moved coherently in the same direction, with the greatest displacement observed at the top of the outer hair cells. As the sound pressure level increased, the vibration peak extended to other structures, particularly the basilar membrane.

At low frequencies, however, the reticular lamina at the apical end of the outer hair cells and the somatic region of the Deiters' cells moved in approximately opposite directions. Additionally, the top and bottom of the outer tunnel exhibited antiphase motions.

Conclusions: These results suggest that the organ of Corti's response at low frequencies reflects localized, outer hair cell-driven motion, while the response at the best frequency is dominated by amplified traveling waves that mask local cellular activity. When sound-induced length change in outer hair cell alters the outer tunnel's circumference of the outer tunnel, they generate fluid pressure oscillations within the tunnel. Due to the incompressibility of cochlear fluid and the forward-propagating traveling wave, these pressure oscillations in the outer tunnel and other fluid spaces produce longitudinal fluid motion within the cochlear partition. This fluid motion, in turn, enhances the traveling wave, thereby increasing cochlear sensitivity.

SU155. Cochlear Fluid Flow Driven by Active Outer Hair Cells

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Background: Fluids in the inner ear are considered stationary because the inner ear fluid system is nearly isolated from other body fluid systems, such as the cerebrospinal fluid and the blood.

Two lymphatic fluids fill three tube-like cavities in the cochlea. The fourth fluid-filled cavity along the tunnel of Corti is unique to mammals (non-mammalian sensory epithelia do not have such an ample extracellular fluid space). Recent studies have shown that the organ of Corti is 3-dimensionally deformed by active outer hair cells, resulting in a change in the cross-sectional area of its fluid-filled space. We hypothesized that active outer hair cells can induce cochlear fluid circulation.

Methods: Experiment) An excitotoxic drug (10 mM kainic acid) that affects afferent synaptic terminals was delivered to the intact round window of young gerbils. A multi-channel electrode placed at the anteroventral cochlear nucleus measured neural signals while the drug diffused along the cochlea. During the drug delivery, sounds at different levels and frequencies were played. Model simulations) The computational model incorporates structural mechanics, hair cell physiology, and fluid dynamics. Three layers of fluid mechanics were essential to this study: interaction between the scala fluid and the cochlear partition, interaction between the Corti fluid, the organ of Corti and active outer hair cells, and advection-diffusion in the scala and Corti fluids.

Results: Sounds affected cochlear drug delivery. At 50 dB SPL, playing sounds had a minimal effect on the drug's effect time. Between 60 and 80 dB SPL, as the sound level increased, the effect time got shorter. Lower frequency was more effective in facilitating drug delivery. At locations apical to the CF location of sounds, often the delivery took equal or longer time when played loud sounds (< 75 dB SPL). Computer model simulations could explain experimental observations such as the accelerating region (located basal to the CF location of sounds) and the transport barrier (near the CF location).

Conclusions: Our measurements and model simulations show that: 1) Active outer hair cells can drive fluid circulation in the cochlea; 2) Two routes construct the fluid circulation: flux toward the apex along the tunnel of Corti and flux toward the base along the scala tympani; 3) A pure tone stimulus generates an accelerated transport region and a transport barrier.

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SU156. Electrically Evoked Displacements of Outer Hair Cells in the Fresh Cadaveric Human Cochlea

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Background: Prestin, a protein crucial for cochlear hair cell electromotility, has been detected using immunohistochemistry in the lateral cell membrane of outer hair cells (OHCs) in human cadaver cochlear tissue, suggesting its structural preservation even postmortem (Rask-Andersen et al., 2010). Little is known about the human organ of Corti (OoC) displacement generated by the electromotile forces from the OHCs.

Methods: Optical coherence tomography (OCT) was used to image and measure vibrations of the cochlear partition (CP) in the apical region of fresh (< 28 hours postmortem) cadaveric human cochleae in response to acoustic stimulation and electrical stimulation. In all cases, the scala vestibuli was fenestrated for OCT and electrode access. The cochlea was immersed and maintained in chilled artificial perilymph media during preparation and measurements.

Acoustical stimulation was performed with an ear canal probe tube speaker. In one case, the incudostapedial joint was disarticulated and a piezoelectric stack was used to drive the stapes. Electrical stimulation was performed by inserting a glass pipette with a 70 μ m diameter tip into the scala media. The return electrode was placed in the dish. Discrete sinusoidal signals over a range of 100 Hz to 20 kHz were used as a stimulus and the OCT measured displacement response of structures within the CP.

Results: Acoustical stimulation OCT vibrometry results indicated CP tuning of approximately 250 Hz in two preparations that were also subject to electrical stimulation. The electrical stimulation frequency response was more broadly tuned, showing a higher frequency cutoff than the acoustic. With acoustic stimulation, the CP structures moved in phase. With electrical stimulation, out of phase displacement between the basilar membrane and reticular lamina was seen only along the axis of the outer hair cells and Deiters' cells. This suggests that the outer hair cell force effect was activated and localized to the OHCs and Deiters' cells axis. These results are consistent with previously reported measurements in animals (Strimbu et al., 2022, Puria et al., 2025).

Conclusions: Prestin's normal electromotile function requires membrane potential, which is absent postmortem. To overcome this limitation, we introduced a scala media electrical probe, which generated an extracellular potential that partially restores OHC motility in our human cadaver preparation and measured with high resolution OCT.

SU157. Immediate Post-Noise and Short-Time Systemic Elevation of Progesterone Promotes Recovery of Cochlear Synapses From Noise-Induced Synaptopathy in Mice

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Background: Female mice are generally less susceptible than males to noise-induced cochlear synaptopathy (NICS). Remarkably, susceptibility to NICS in females varies through the estrous cycle. Females exposed to noise in the luteal phase (metestrus and diestrus) exhibit reduced synapse loss, while females exposed to noise in the follicular phase (proestrus and estrus) exhibit synapse loss like that in noise-exposed males (Hu et al, ARO-Meeting-2017). Reduced susceptibility to NICS correlates with elevated systemic progesterone (P4) levels, the dominant hormone in the luteal phase, but not with estrogen (E2), the dominant hormone in the follicular phase. To test the hypothesis that P4 could protect against NICS or facilitate recovery from NICSs, P4 was chronically administered via an implanted pellet or acutely administered via subcutaneous injections at various times, pre-noise or post-noise, in CBA/CaJ mice.

Methods: 12-14-week-old males or ovariectomized (OvX) females were exposed to 100 dB SPL, 8-16 kHz octave band noise for 2 hours. Auditory brainstem response (ABR) and DPOAEs were measured at 8, 16, and 32 kHz at three times: pre-noise, post-noise day 1 (PND1), and PND14 to assess baseline thresholds, temporary threshold shifts, and recoveries in a within-subject design. For chronic administration, a P4-pellet (P4P) was implanted subcutaneously, with ABRs measured pre- and post-implantation/pre-noise. For acute administration, water-soluble P4 (P4W) or P4 in sesame oil (P4O) was injected 2-hr pre-noise and/or immediately pre-noise (PrN2, PrN0), and/or immediately or 2-hr post-noise (PN0, PN2). Changes in ABR wave-I

amplitudes at PND14 were quantified as amplitude decline relative to pre-noise. To quantify synapses/inner hair cell, postsynaptic densities, presynaptic ribbons, and hair cells were visualized using anti-PSD95, anti-Ribeye/CTBP2 and anti-myosin antibodies, respectively, in organ of Corti wholemounts at 8, 16, and 32 kHz locations in mice euthanized at PND14.

Results: In P4P OvX females, pre-noise ABR wave-I amplitudes were slightly reduced. Recovery of amplitude on PND14 was not significantly better than nonimplanted controls; synapse counts remained lower than in non-noise exposed controls although slightly higher than nonimplanted controls. In acute P4W or P4O groups, post-noise recovery of ABR amplitudes and synapse numbers were better than the saline control, with no difference between males and OvX females. In both males and OvX females, P4O administered at PN0-only showed similar results to P4O at pre- plus post-noise and better recovery than PrN-only in amplitude recoveries and synapse counts. We did observe increased risk of PTS associated with P4 administration.

Conclusions: P4 ameliorates NICS assessed by ABR amplitude and synapse counts. This appears to be due primarily to facilitating post-noise recovery rather than protection during noise. Chronic P4 delivered from an implanted pellet provides relatively little recovery from NICS relative to acutely-delivered P4 administered by injection following noise exposure. (Supported by NIDCD R01 DC015790)

SU158. Transient Transfection of Fibroblasts to Produce Bdnf on the Surface of Cochlear Implants

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Background: After cochlear implantation, connective tissue is formed around the implanted electrode array. Despite many different efforts to reduce connective tissue formation, some cells will always remain on the electrode surface. Furthermore, the function of a cochlear implant (CI) is relying on the presence of intact spiral ganglion neurons (SGN). Survival of SGN can be supported by the application of neurotrophic factors such as brain-derived neurotrophic factor (BDNF). The aim of our approach is to modify existing fibroblasts on the surface of a CI electrode array to produce and deliver BDNF. This production on the electrode surface could also provide a directional cue in case new neurites are formed by SGN.

Methods: Two recombinant vectors coding for td-Tomato and BDNF (5.6 and 8.8 kbp) were used to transfect NIH/3T3 cells. The plasmids were complexed using different amounts of branched 25 kDa polyethyleneimine (PEI). Complex formation (size and surface charge) was investigated using a zetasizer for different ratios of PEI and DNA. The substances alone as well as the complexes were tested regarding cytocompatibility using cell viability tests. For transfection, the cells were incubated with the complexes for 3 hours before further cultivation. Transfection efficiency was determined 24 hours after starting the incubation by comparing cells exhibiting red fluorescence to the total number of cells.

Results: Complexes between DNA and PEI were successfully formed. Up to an amount of PEI of 1000 ng per well, the complexes appeared to be cytocompatible. Transfection efficacy at 24 hours after start of the incubation was below 1% for the large vector and close to 10% with the

smaller vector with the highest values for equal amounts of DNA and PEI in the solution. The complexes could be incorporated into polymeric coating that can be used for cochlear implant electrode arrays.

Conclusions: With the smaller vector, a system to transfect fibroblasts to produce and deliver BDNF is available that can potentially be combined with CI electrodes. This system can now be used to investigate further questions such as release from the coating, duration of transfection etc.

SU159. Clustering Analysis Reveals Distinct Macrophage Phenotypes Following Excitotoxic Injury in the Zebrafish Lateral Line

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Background: Macrophages exhibit diverse morphological and motile features that reflect distinct responses to cochlear injury and subsequent synaptic repair. Recent studies in mice demonstrate that macrophages are recruited to the cochlea after moderate-intensity noise exposure, but the dynamic transitions between macrophage states during excitotoxic injury remain poorly understood. Real-time visualization of macrophage-synapse interactions in the intact mammalian inner ear is challenging, but the zebrafish lateral line provides a powerful model to enable fluorescent labeling and time-lapse imaging of macrophage behavior in vivo. Here, we employed live imaging and unsupervised clustering of macrophage tracks and features to identify and characterize discrete macrophage phenotypes following excitotoxic injury.

Methods: 6-7 day old transgenic zebrafish expressing a red fluorophore in macrophages (tg(mpeg1:tdtom)) and either GFP in afferent neurons (tg(neurod:GFP)) or a cytosolic targeted Ca²⁺ indicator (tg(hsp70:GCaMP6s:sill1)) were imaged with a spinning disc confocal microscope. Fish were paralyzed with α -bungarotoxin and mounted in an imaging chamber before visualizing the neuromast hair cells and afferent neurons. After obtaining baseline images, fish were exposed to 100-300 μ M AMPA for 15-30 minutes to simulate excitotoxic injury and were then rinsed with embryo media. Time-lapse imaging followed for 2-3 hours, and results were compared to control fish without AMPA exposure. Automated tracking (FIJI-Trackmate) and unsupervised clustering analysis (CellRomeR) were utilized to compare morphologic (area, circularity) and motility (speed, directional change rate) features to identify phenotypic clusters unique to each condition.

Results: Cluster analysis revealed distinct macrophage phenotypes with different prevalence in the control and AMPA-exposed conditions. The control condition was composed primarily of macrophages that were larger and slower without significant directional changes, which was consistent with a “surveillance” phenotype. In the AMPA-exposed group, there were multiple distinct phenotypes with a portion of macrophages adopting the “patrolling” phenotype but a larger proportion that were smaller and faster with a high directional change. Such behavior is consistent with an “activated” phenotype. These experiments are ongoing.

Conclusions: Our findings demonstrate that excitotoxic injury results in shifts between distinct macrophage phenotypes in the zebrafish lateral line. The results highlight the utility of clustering analysis in identifying discrete macrophage subtypes in vivo and for linking dynamic morphologic and motility features to injury. Further work will involve implementing a similar

cluster analysis paradigm for the lateral line imaged with light sheet microscopy after excitotoxic injury.

SU160. Investigating the Role of Neogenin in Cochlear Synaptopathy

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Background: Synapses between cochlear hair cells and Type 1 auditory nerve fibers are the most vulnerable to acoustic trauma, leading to permanent synaptopathy despite recovery of temporary threshold shifts. Previous therapeutic interventions in mouse models have demonstrated a limited window for synaptic recovery. Treatments, such as neurotrophins, administered before or immediately after noise trauma have shown a protective rather than a regenerative effect. However, an antibody targeting Repulsive Guidance Molecule A (RGMA) restored synapses for up to 1 week after noise exposure, suggesting a regenerative effect on the synapse. RGMA is involved in axonal guidance through interactions with neogenin1 (Neo1), a cell surface receptor expressed on auditory nerve fibers. Epigenetic barriers can restrict synaptogenesis in the adult sensory nervous system, limiting the restoration of the cochlear afferent synapse in the noise-exposed inner ear. Here, we hypothesize that RGMA-Neo1 interactions inhibits synaptic repair by facilitating the nuclear translocation of the Neo1 intracellular domain (Neo-ICD), which mediates epigenomic changes after acoustic trauma.

Methods: We developed clonal Neo-HA Neuro-2a cell lines and generated Neo1 and Neo-ICD EGFP tagged constructs to evaluate regulated intramembrane proteolysis and subcellular localization of fragments using immunoblotting and immunofluorescence under different pharmacological conditions. Nuclear activity and chromatin binding of Neo-ICD was assessed in-vitro using Cut and Run assays. To evaluate functional recovery of synapses, we inhibited Neo-ICD cleavage in an ex-vivo kainic acid (KA) excitotoxicity model of cochlear synaptopathy and quantified synaptic counts.

Results: Neo1 undergoes endogenous stepwise proteolysis to release the Neo-ICD in-vitro. We observed that intramembrane proteolysis is mediated by γ -secretase and Neo-ICD undergoes rapid degradation by the proteasome. Nuclear translocation is tightly regulated where nuclear export, but not nuclear import, determines steady state localization. Cut and Run assays revealed that Neo-ICD binds to chromatin with co-localization to histone markers. Preventing intramembrane cleavage of Neo-ICD in ex-vivo KA models showed a dose-dependent effect to regenerate cochlear synapse counts.

Conclusions: Our findings identify neogenin1 as a potential epigenetic regulator of chromatin accessibility and may provide a novel therapeutic strategy to extend the therapeutic window for synaptogenesis in the cochlea.

SU161. Using Simulations and Non-Stationary Fluctuation Analysis of Synaptic Currents to Uncover the Properties of Cochlear AMPA-Type Glutamate Receptors: Ca²⁺ Permeable and Impermeable

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Background: AMPA receptor/channels are concentrated in specific regions of synapses called postsynaptic densities (PSDs) where they mediate fast neurotransmission. PSDs vary in size among synapse type, differing in number, spatial density, and composition of subunits. For spiral ganglion neurons (SGNs) contacting inner hair cells, PSD composition heterogeneity may in part determine sound response properties. However, the number of AMPARs within single PSDs, their spatial organization, and their molecular heterogeneity are currently unknown.

Nonstationary fluctuation analysis (NSFA) has been used to quantify the number of receptors (N) and their unitary currents (i) in synapses of the CNS. By recording and analyzing excitatory postsynaptic currents (EPSCs), NSFA can characterize AMPAR properties. Yet while NSFA outputs a single estimate for i, unitary currents depend on AMPAR subunit composition. Depending on the absence or presence of the GluA2 subunit, AMPARs are calcium permeable (CP-AMPARs) or calcium impermeable (CI-AMPARs), respectively.

To extract distinct i estimates for CP-AMPARs and CI-AMPARs and their relative abundance, here we report on a method of analysis taking advantage of IEM-1925, a selective CP-AMPAR blocker. We will also share the parameters of a simulation matching the kinetics of physiological EPSCs. Finally, we will compare NSFA results on simulated EPSCs (sEPSCs) to NSFA on vesicular EPSCs (vEPSCs), EPSCs evoked by glutamate uncaging (uEPSCs), and vEPSCs of cells treated with IEM-1925.

Methods: Explants of the organ of Corti from P15-P17 C57BL/6 mice were used for patch-clamp recordings. uEPSC recordings were generated via rapid homogeneous uncaging of glutamate on the entire PSD. vEPSCs were recorded before and after treatment with IEM-1925. EPSC simulations and NSFA tools were scripted in Python.

Results: To reproduce the ranges and distributions of amplitudes and kinetics observed in vEPSCs, the simulations required that each EPSC be generated from variable N and [glutamate], defined by normal distributions, fit to an ensemble of physiological data: $\mu=2200$, range=50–3422; and $\mu=2.5\text{mM}$, range=1–5.8mM, respectively.

It is unclear how robustly NSFA can extract N and i in recordings from synapses with such heterogeneous EPSCs. For sEPSCs, some permutations of NSFA produced accurate estimates of i. However, information on N and Popen remain obscure.

For sEPSCs and uEPSCs, where each channel sees glutamate simultaneously, the pattern of EPSC amplitudes and kinetics are similar, but both differ from patterns observed for vEPSCs. Inclusion of a simple model of glutamate diffusion may in part explain these differences.

NSFA revealed significantly lower N and i for IEM-1925 versus control. By comparing mean i between EPSCs recorded under control vs. a saturating concentration of IEM-1925, we extracted i for CP-AMPARs and CI-AMPARs and their relative proportions.

Conclusions: Simulations of EPSCs recorded from SGNs can validate NSFA on physiological data to extract essential channel properties in support of pre-clinical translational research.

SU162. Single-Cell and Spatial Transcriptomic Analysis of Noise-Induced Cochlear Synaptopathy

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Background: Noise-induced hearing loss (NIHL) may arise in part from cochlear synaptopathy, the loss of ribbon synapses between inner hair cells and spiral ganglion neurons (SGNs). This process, thought to be driven by glutamate excitotoxicity, occurs even in the absence of hair cell loss and disrupts the fidelity of auditory signaling. Synaptopathy has been studied as a cause of auditory dysfunction, and has demonstrated tonotopic differences in synapse loss after noise exposure. However, the cellular heterogeneity of SGN responses and the molecular mechanisms that mediate synaptic degeneration and repair remain incompletely defined.

Recent evidence has identified the molecular subtypes of SGNs, which correlate with their electrophysiological properties and susceptibility to excitotoxic injury (Shrestha et al. 2018). There are three Type 1 SGN subtypes (1A-C), with Type 1C being the most susceptible to noise-induced synaptic loss. We aim to determine the underlying molecular responses in noise-induced hearing loss and cochlear synaptopathy using single-cell and complementary RNA in situ hybridization for spatial relationships. We hypothesize that there are SGN-specific and tonotopic-regional differences in synapse related genes after noise exposure.

Methods: A two-tiered approach was used to investigate transcriptional changes after glutamate excitotoxicity and noise-induced cochlear synaptopathy. First, an in vitro 3D cochlear explant model of neonatal mice (P2-3) were exposed to a glutamate agonist or glutamate agonist and neurotrophins to induce synapse regeneration. Following treatment, explants underwent single cell RNA sequencing and computational analysis.

To validate transcriptomic findings in vivo, an established mouse model for noise induced synaptopathy was used. The synaptopathy phenotype was confirmed using ABR/DPOAE measurements and synapse quantification on immunohistochemistry using anti-CtBP2 and anti-GluR2 antibodies. Cochleae were harvested 24 hours post noise-exposure and tonotopically microdissected. Spatial expression of candidate genes was examined using RNA in situ hybridization (RNA-ISH) in a whole mount preparation in the 11.33 kHz and 32 kHz regions. Machine learning-assisted three-dimensional imaging analysis was used for quantification.

Results: Single-cell RNA sequencing of cochlear explants following glutamate excitotoxic injury and neurotrophin exposure yielded high-quality cells across conditions. Clustering identified populations of hair cells and spiral ganglion neurons identified using their canonical markers. After glutamate agonist exposure, there was an increase in the expression of glutamate receptor subunit mRNA and an even greater increase after NT3 exposure. In vivo validation using RNA-ISH in noise-exposed mice confirmed spatially distinct increases in glutamate receptor subunit mRNA by SGN subtype and by tonotopic region.

Conclusions: These data suggest that subtypes of spiral ganglion neurons susceptible to synaptic loss due to noise trauma, respond in turn by generating more synapses. These findings provide a greater insight into the mechanisms that underlie synaptic loss and recovery which could garner targeted treatments for noise-induced hearing loss.

SU163. Glu2Py as a Novel Neurotrophic Agent for Inducing Neuritogenesis in Spiral Ganglion Cultures

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Background: Cochlear synaptopathy, involving the loss of afferent ribbon synapses between cochlear hair cells and spiral ganglion neurons (SGNs), is a leading cause of sensorineural hearing loss and continues to present a major clinical challenge due to the lack of effective therapeutic options. The development of neurotrophic compounds that can promote spiral ganglion (SG) neurite outgrowth and enhance neuronal survival is critical for advancing hearing restoration strategies. Glucosyl-2-pyrrolopyrimidinone (Glu2Py), a novel pharmacologic inhibitor of Misshapen Kinases, has shown potential neurotrophic activity in preliminary studies. However, its specific effects on auditory neurons and nerve fibers remain to be systematically characterized.

Methods: We evaluated the neurotrophic effects of Glu2Py (1, 5, and 10 μ M) using multiple experimental models, including SH-SY5Y human neuroblastoma cells, cochlear spiral ganglion explants, and dissociated SGN cells from postnatal day 3.5 CD1 mouse pups. SH-SY5Y cells were treated with Glu2Py for either 24 or 48 h followed by 48-h recovery periods, while SG tissues were exposed to Glu2Py for 24-48 h depending on the experimental design. Cell morphology measurements and neurite length analysis were performed using fluorescence microscopy and ImageJ software with NeuronJ plugin. Statistical analysis employed appropriate parametric and non-parametric tests based on data distribution.

Results: Glu2Py demonstrated significant dose-dependent and time-dependent neurotrophic effects across all models. In SH-SY5Y cells, 24-h treatment showed concentration thresholds with increased neurite-to-cell outgrowth at 1 (1.00 ± 0.42), 5 (1.23 ± 0.10), and 10 μ M (1.52 ± 0.05) versus untreated cells (0.56 ± 0.14). Neurite outgrowth directly affected mean cell area, confirming treatment effects. Treatments at 1, 5, and 10 μ M significantly increased cell areas (547.95 ± 246 , 599.51 ± 326.21 , and 572.61 ± 296.61 μ m², respectively, $p < 0.0001$) compared to controls (341.49 ± 170.16 μ m²). In 48-h treatment groups, mean area increased at 5 μ M (601.43 ± 308.64 μ m², $p < 0.01$) and 10 μ M (1183.48 ± 442.81 μ m², $p < 0.0001$) versus controls (423.67 ± 111.31 μ m²), while 1 μ M showed no significant effect. Spiral ganglion explants demonstrated robust concentration-dependent neurite outgrowth, with highly significant increases at 5 μ M (94.21 ± 31.39 μ m) and 10 μ M (102.63 ± 33.04 μ m) compared to controls (65.44 ± 14.55 μ m, $p < 0.0001$), while 1 μ M showed no significant effect (59.51 ± 21.91 μ m). Remarkably, separated spiral ganglion cells exhibited enhanced sensitivity, showing significant neurite extension at all concentrations: 1 μ M (81.19 ± 17.62 μ m), 5 μ M (77.55 ± 33.62 μ m), and 10 μ M (99.90 ± 20.01 μ m) compared to controls (53.54 ± 17.92 μ m, $p < 0.0001$). Differential

responses between explant and separated cultures suggest tissue architecture and cellular microenvironment significantly influence compound efficacy and cellular responsiveness.

Conclusions: Glu2Py exhibits potent neurotrophic activity with particular efficacy in promoting SGN outgrowth. The compound shows time-dependent effects in a human neuronal model and potency in primary tissue explants and dissociated SGNs. These findings establish Glu2Py as a promising therapeutic candidate for cochlear neuropathy and synaptopathy, warranting further investigation in ototoxicity protection and regenerative sensorineural hearing loss treatment applications.

SU164. Daily Dynamics of the Electrode-Tissue Interface in Cochlear Implants With and Without Steroid-Eluting Electrodes

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Background: Cochlear implant electrode impedance provides insight into inner ear tissue responses. After implantation, impedance typically rises due to protein adsorption and inflammatory tissue changes, then several changes occur after the onset of electrical stimulation. Excessive impedance growth is linked to fibrotic encapsulation of the electrode and can negatively impact device performance. Steroid interventions (e.g. topical or eluting dexamethasone) have been explored to mitigate these foreign body responses and impedance increases. However, the day-to-day impedance dynamics in the immediate remain poorly characterized. This study aimed to monitor daily impedance changes and their resistive vs. polarization subcomponents in cochlear implant recipients with and without sustained steroid delivery, and to relate these changes to device usage patterns.

Methods: A prospective observational design was used to analyze daily impedance telemetry (morning and night) in cochlear implant recipients. Four groups were studied based on implant type and steroid administration: (1) CI612 electrodes with no dexamethasone application, (2) CI612 electrodes with a single intraoperative topical dexamethasone application, (3) CI632 electrodes with the same topical dexamethasone treatment, and (4) CI632 electrodes incorporating a self-eluting dexamethasone polymer for sustained release. Impedance was measured daily from the day of surgery through the post-activation period using standard clinical telemetry. To dissect impedance components, we calculated the resistive and polarization subcomponents. Patients' device data-logging records (hours of use per day) were also collected in a subgroup to examine the influence of daily cochlear implant use on impedance fluctuations.

Results: Daily impedance monitoring revealed consistent temporal patterns across patients. As expected, impedance increased during the early postoperative period and stabilized over time. Significant differences were observed across electrode types and dexamethasone delivery methods. After activation, clear diurnal fluctuations emerged, primarily driven by changes in the polarization component, while the resistive component remained stable. The self-eluting dexamethasone electrode exhibited the most stable profile, significantly reducing day-to-night changes and flattening the polarization impedance curve. Differences in the resistive subcomponent were minimal across groups. Notably, patients with greater daily CI use showed larger diurnal impedance shifts, suggesting that electrical stimulation modulates the electrode-tissue interface.

Conclusions: Our findings demonstrate that the polarization component of electrode impedance is highly sensitive to changes in cochlear implant (CI) stimulation and is significantly modulated by anti-inflammatory interventions. Dexamethasone, particularly when delivered via a self-eluting electrode array, not only attenuates the post-surgical rise in impedance but also reduces day-to-day variability, leading to a more stable electrode–tissue interface. In contrast, the resistive component remains largely unaffected by both steroid intervention and electrode type. From a clinical perspective, daily impedance monitoring offers a high-resolution window into cochlear healing and tissue response. When combined with targeted steroid delivery, this strategy may enable earlier detection of abnormal biological responses and support more personalized postoperative management.

SU165. Feasibility of Endoscopic Microneedle-Guided Round Window Membrane Perforation and Perilymph Sampling in Human Cadaveric Temporal Bones

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Background: Diagnostic and therapeutic interventions for inner ear disorders remain limited by the lack of safe, controlled access to the perilymphatic space. Haystack Medical has developed a microneedle-endoscope system designed for minimally invasive round window membrane (RWM) perforation and fluid aspiration under direct visualization.

Methods: Hollow microneedles (outer diameter 100 μm , lumen diameter 35 μm) were fabricated by two-photon lithography and mounted onto polyimide tubing. A refined deployment tube (23 Ga) enabled endoscopic visualization and precise positioning adjacent to the RWM. Cadaveric human temporal bones ($n=7$) were used to evaluate procedural feasibility. Following endoscopic RWM perforation, perilymph aspiration was performed using a syringe pump at $\sim 1 \mu\text{L}/\text{min}$. Aspirated volumes were quantified microscopically via droplet measurement.

Results: Controlled, reproducible RWM perforation was achieved in all specimens (100% success, $n=7$) without tearing or enlargement of the membrane. Aspiration was successful in all cases, with mean volume 0.89 μL (SD 0.20 μL), slightly below DI water controls (1.0 μL). Needle integrity was preserved after each use, and no uncontrolled RWM damage was observed. Some aspirates contained visible blood contamination, likely attributable to cadaveric conditions.

Conclusions: Haystack Microneedles enabled consistent, visually guided perforation and reliable perilymph aspiration in human cadaveric models. Achieved aspiration volumes are sufficient for biomarker analyses, demonstrating feasibility of minimally invasive diagnostic sampling. These Phase I outcomes establish a strong foundation for clinical translation and regulatory preparation.

SU166. Development of a Drug-Induced Ototoxicity Model Using Inner Ear Organoids and Investigation of Protective Mechanisms

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Background: Ototoxic drugs such as aminoglycoside antibiotics and cisplatin are widely used in clinical practice

but are a major cause of irreversible hearing loss, primarily through damage to cochlear hair cells.

Current ototoxicity studies primarily rely on animal models or two-dimensional cultures, which fail

to adequately reproduce the structural and functional complexity of the human inner ear. Human pluripotent stem cell–derived inner ear organoids represent a physiologically relevant platform with the potential to model both auditory development and drug-induced damage. The aim of this study is to establish an inner ear organoid–based ototoxicity model and to explore protective mechanisms through the modulation of key signaling pathways, including Wnt, PI3K/PTEN, and

YAP

Methods: Human embryonic stem cells and induced pluripotent stem cells were differentiated into inner ear

organoids using protocols established in our laboratory. This process involved the induction of otic placode formation, vesicle development, and sensory epithelium differentiation. Organoids are

characterized by immunostaining and molecular assays for lineage-specific markers, including ATOH1, POU4F3, and MYO7A (hair cells), as well as SOX2 and PROX1 (supporting cells). To model

ototoxic injury, organoids will be treated with aminoglycoside antibiotics and cisplatin, followed by

evaluation of cell survival, ROS generation, and morphological changes using immunostaining and

LDH assays. Protective strategies will be tested by applying small molecules, including the Wnt activator CHIR99021, the PTEN inhibitor VO-OHpic, and the ROS scavenger N-acetylcysteine.

Transcriptomic profiling via RNA-seq will be performed to identify molecular pathways involved in

damage and recovery.

Results: Preliminary experiments have focused on directing human pluripotent stem cells toward inner ear

organoid differentiation. Organoid formation efficiency is currently being systematically evaluated

across multiple cell lines, and immunostaining is being used to quantify the proportion of organoids expressing otic lineage markers. Ototoxic drug treatment has not yet been initiated; protocols for exposure to aminoglycoside antibiotics and cisplatin are under development. Comprehensive quantitative analyses will be conducted in the next phase, and results will be presented at the meeting.

Conclusions: This study highlights the potential of human pluripotent stem cell–derived inner ear organoids as

a physiologically relevant model for investigating drug-induced ototoxicity. Modulation of Wnt, PI3K/PTEN, and YAP signaling pathways is expected to provide novel insights into protective mechanisms for auditory hair cells. Such an approach may contribute to the establishment of a precise preclinical drug-screening platform and facilitate the development of innovative therapeutic strategies aimed at preventing or reducing hearing loss induced by ototoxic agents.

SU167. A Novel Device for Atraumatic Perilymph Sampling

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Background: A promising approach to investigating cochlear physiology is the analysis of perilymph, a sodium-rich extracellular fluid present in the cochlea and vestibular system with interaction with key auditory structures. Because of its anatomically challenging location and the immediate risk of irreversible hearing loss following cochlear injury, perilymph sampling in humans is currently only feasible during surgical procedures that are already expected to result in hearing loss, such as cochlear implantation or removal of vestibular schwannoma. In contrast, animal models allow the evaluation of innovative approaches for perilymph collection. Several techniques are available for obtaining perilymph from animal models, varying in their degree of invasiveness and potential to cause irreversible damage. The choice of methodology should be guided by the specific scientific objective, with the aim of collecting perilymph as atraumatically and uncontaminated as possible to ensure its validity as a diagnostic tool.

Methods: In three pigs (*Sus scrofa domestica*), surgical access to the inner ear was established, and the round window membrane was exposed. A novel sampling device was advanced through the round window membrane to collect perilymph. In two animals, auditory brainstem responses (ABRs) were recorded both preoperatively and one week postoperatively to assess potential changes in auditory function resulting from the procedure. Following functional assessment, the inner ears were harvested and subjected to high-resolution micro-computed tomography (micro-CT) analysis. To confirm that the collected fluid was indeed perilymph, the presence of cochlin was verified using an enzyme-linked immunosorbent assay (ELISA).

Results: After surgical exposure of the round window, perilymph was successfully collected using the novel device. In three samples, a volume of 3–5 µL was obtained, in which the presence of cochlin was clearly confirmed via ELISA. The structural integrity of the cochlea

remained largely preserved, as shown in the micro-CT data. In the two animals monitored for one week postoperatively, minor shifts in hearing thresholds were observed in some instances, with an average change of 5 dB across all frequencies.

Conclusions: Atraumatic collection of perilymph using the novel device was successfully demonstrated in a large animal model. The procedure required minimal training for surgeons and allowed the collection of sufficient volumes for diagnostic and analytical purposes. Although slight shifts in hearing thresholds were observed, these were minimal and cannot be conclusively attributed to the device itself, as the surgical procedure inherently carries a risk of minor threshold changes. Micro-CT imaging revealed only limited structural alterations at the round window membrane, suggesting favorable conditions for subsequent healing. To further assess recovery and long-term outcomes, studies with extended follow-up periods are warranted.

SU168. Perilymph-Sampling: A Translational Journey

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Background: Translational cochlear implantation, gene therapy and pharmacological distribution strategies require robust animal models to effectively mimic human cochlear anatomy and physiological responses, as well as a similar genetic profile. The pig has emerged as an ideal large animal model due to its anatomical and physiological similarities to the human inner ear. Here, we summarize recent advancements and outcomes from out pig-based cochlear implantation, gene and drug delivery studies providing crucial translational insights.

Methods: We established and continually refined a cochlear implantation surgical protocol in pigs designed to replicate human cochlear anatomical dimensions. This approach facilitates the examination of trauma induced by electrode insertion and optimizes implantation techniques for clinical translation. Drug delivery studies, conducted using various infusion methods with and without CE-certified catheters, provided critical insights into fluid dynamics within the large cochlea.

Results: Utilizing robotic-assisted cochlear implantation, we observed significant reductions in electrode impedance and improved hearing preservation compared to conventional manual insertions, highlighting the potential of robotic assistance to enhance implantation outcomes and reduces intracochlear trauma. We developed and implemented comprehensive perilymph sampling protocols in the pig, including traumatic and atraumatic round window membrane sampling, stapedotomy and stapes fenestration, semicircular canal opening and apical sampling. These methods enabled detailed proteomic and transcriptomic characterization of perilymph before and after cochlear implantation. Notably, our data represents the first description of porcine cochlear expression profiles, providing unique opportunities for comparative analysis with human perilymph expression data. Additionally, we assessed cochlear distribution and off-target effects of various viral vectors, particularly adeno-associated viruses (AAV). Our results from gene delivery studies demonstrated successful cochlear transduction without requiring fenestration, suggesting that intracochlear administration of AAVs is feasible and effective

across the entire cochlear length. However, distribution patterns varied considerably between the chosen vector and the methodology of injection.

Conclusions: Data generated from porcine studies offer a high degree of translationality to clinical application. Differences in transduction from gene delivery studies correlate indirectly with functional outcomes in ongoing otoferlin gene therapy studies, emphasizing the importance of vector selection and delivery precision.

Collectively, these findings underscore the pig's model utility in translational auditory research, providing pivotal insights that advance multiple fields from cochlear implantation to gene therapy strategies for hearing loss treatment.

SU169. Histamine Receptors in the Auditory Midbrain and Thalamus of the Mouse

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Background: The central histaminergic nervous system, originating from the tuberomammillary nucleus (TMN) of the hypothalamus, widely innervates almost the whole brain including the midbrain and thalamus. Brain histamine regulates diverse physiological functions, including wake and arousal states, forming part of the ascending reticular activating system. While the modulatory role of histamine in the brain, particularly in the cerebral cortex, is widely explored, very little is known about its function in the auditory system; in particular, histamine's role in the auditory midbrain (inferior colliculus: IC) and auditory thalamus (medial geniculate body: MGB) has been largely unexplored

Methods: Therefore, to assess histaminergic innervation of these auditory structures, we neuroanatomically and physiologically assessed IC and MGB neurons for functional histamine receptors. We immunohistochemically stained for histamine receptor subtypes (H1R, H2R, H3R) in both the IC and MGB. In addition, we employed in vitro whole-cell patch-clamp to record from IC and MGB neurons in response to bath application of histaminergic agonists.

Results: From our studies, we find a unique distribution of histamine receptors in the IC and MGB of the mouse that correlates with the responsiveness of neurons to histaminergic agonists, which in general act to depolarize auditory neurons

Conclusions: Overall, our data suggests that histamine can selectively heighten the responsiveness of auditory thalamic and midbrain structures to acoustic signals and highlights the overlooked role of histamine in these structures of central auditory processing

SU170. Acetylcholine Release in the Inferior Colliculus During a Stimulus-Specific Adaptation Paradigm

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Background: Stimulus-specific adaptation (SSA) is a neural process that supports novelty detection by selectively diminishing responses to repeated sensory stimuli. Detection of novel

sounds is important for survival and communication, but the neural mechanisms underlying novelty detection in the central auditory system remain largely unclear. The inferior colliculus (IC) serves as a major hub for auditory information integration and is the first auditory subregion to exhibit SSA in the ascending auditory pathway. Additionally, exogenous application of acetylcholine (ACh) reduces SSA in the IC by enhancing responses to repeated sound stimuli (Ayala and Malmierca, 2015). Previous anatomical studies indicate the IC receives dense cholinergic innervation from the pedunculopontine tegmental nucleus (PPN), a key component of the reticular activating system that influences arousal, attention, and sensory processing. Several studies have indicated that PPN neurons respond to sounds and rapidly adapt to repeated sounds, but how cholinergic signaling from the PPN shapes responses in the auditory system remains largely unknown. We hypothesize that cholinergic signaling from PPN projections to the IC modulates SSA in the IC. As the first step in testing this hypothesis, here we examine how novel and repeated sounds shape ACh release in the IC.

Methods: We are currently investigating ACh release in the IC using in vivo fiber photometry in mice. Adult P60-P90 B6.CAST-Cdh23Ahl⁺ mice, balanced for sex, are injected in the IC with an adeno-associated virus encoding the fluorescent ACh sensor, GRAB-ACh3.0 (GPCR-Activation-Based-ACh), and implanted with an optical fiber in the IC. Several weeks later, mice are head-fixed in a sound-isolation booth and exposed to a pure-tone oddball stimulation paradigm, consisting of standard tones at 70 dB SPL for 200 ms for 90% of trials pseudorandomly intermixed with deviant tones at 70 dB SPL for 10% of trials. Interstimulus intervals vary by 2-5s for a 30-minute recording period. Fluorescent GRAB-ACh3.0 signals are analyzed to determine the amount of ACh release evoked by the standard and deviant tones.

Results: Thus far, we have successfully expressed AAV9-hSyn-GRAB_ACh3.0 in the IC and confirmed that GRAB-ACh3.0 fluorescent signals change in response to novel acoustic stimuli. Experiments to assess how ACh release in the IC changes during SSA stimulus paradigms are currently underway.

Conclusions: This project aims to elucidate how acoustic stimuli influence the release of ACh in the IC and the role of cholinergic signaling in the IC in SSA. We expect our results will show that novel sound stimuli elicit more ACh release in the IC than repeated sound stimuli. We will next use optogenetic tools to manipulate cholinergic PPN projections to the IC to test how inhibiting and enhancing these projections alters IC neuron responses to novel and repeated stimuli.

SU171. Decoding Single and Multi-Neuron Activity in Inferior Colliculus for Speech Digits in Natural Environmental Noise

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Background: Inferior colliculus (IC) is one of the brain's crucial auditory processing and integration centers. Previous results suggest that IC may play a key role in the representation of spectral and temporal modulation statistics (Zhai et al., 2020), and computational models of IC have recently been shown to predict how different background sounds impact speech recognition accuracy (Clonan et al. 2025). Although previous work in IC has examined on how simple sounds, such as tones, are segregated from background noise, how IC contributes to the encoding

and segregation of speech in the presence of natural background sounds is not yet well understood.

Methods: This project aims to characterize how spectrum and modulation cues in natural background sounds influence the representation of speech in IC activity of un-anesthetized rabbits. Using an stimulus paradigm based on a previous study in human listeners (Clonan et al 2025), we delivered speech digits (0 to 9) embedded in 11 original (OR) natural background sounds and perturbed variants where we either preserved the spectrum (spectrum matched, SM) or modulation (modulation matched, MM) statistics of each sound at 0 dB signal-to-noise ratios (SNR). We then use a decoding approach to classify digits based on single neuron recordings from IC was obtained while animals listen to these speech-in-noise stimuli. Using Spike Distance Metric classifiers that analyze spike trains from individual or multiple single neurons at different time-scales we evaluated how IC responses contribute to speech identification.

Results: As expected, classification accuracy improved with increasing number of neurons. While the single- and multi-neuron digit classification accuracy for clean speech was often very high (~99%), accuracy for speech-in-noise varied widely depending on the background. Accuracy varied substantially from background to background and was generally highest for SM backgrounds and lowest for MM. We observe that the discriminability of spike trains peaks at frequency bands with center frequency of ~4 Hz and ~100 Hz, corresponding to word rates and periodicity pitch frequencies of the speech vocalizations.

Conclusions: The general classification accuracy trends for OR, SM and MM backgrounds are correlated with those observed in human behavior (Clonan et al 2025) suggesting that spectrum and modulation statistics of each background influence decoding accuracy. Furthermore, the results indicate that rhythm (~4 Hz) and periodicity pitch (~100 Hz) bands may play a critical role in the subcortical representation of speech. Funded by NIH (R01DC020097).

SU172. Characterization of Subcortical Presynaptic Inputs to Inferior Colliculus Subdivisions

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Background: The inferior colliculus (IC) is a mandatory relay station in ascending auditory pathways and plays an important role in sensory integration. Classically, the IC is divided into core (lemniscal) and shell (non-lemniscal) regions, which differ in sound response properties and connectivity. While projections to the core, or central nucleus (CIC), are well established across species, inputs to the shell, or dorsal (DCIC) and external (ECIC) cortices, remain understudied. Furthermore, our recent work showed that rostral and caudal ECIC subregions relay ascending inputs to distinct thalamic targets with different latencies, raising the possibility that they may convey specialized information from distinct sources.

Methods: In this study, we quantified ascending inputs to the IC subdivisions by performing monosynaptic retrograde tracing from CIC, DCIC, rostral ECIC, and caudal ECIC using fluorophore-conjugated wheat germ agglutinin in mice. Coronal brain sections were aligned to a brain atlas for systematic quantification of input cell distributions.

Results: Preliminary results show distinct subcortical input patterns across subregions. Inputs to CIC were largely consistent with previous literature, with dense projections from the ventral cochlear nucleus, intermediate and ventral nuclei of the lateral lemniscus, and the lateral superior olive. In contrast, shell regions received broader inputs, including strong projections from the paralemniscal nucleus, dorsal and intermediate nuclei of the lateral lemniscus, and additional contributions from motor-related areas in the medulla, pons, and superior colliculus. Notably, rostral ECIC received a higher fraction of input from the ventral cochlear nucleus than caudal ECIC or DCIC, potentially supporting its short-latency sound responses.

Conclusions: Ongoing experiments will further compare input patterns across these IC subdivisions. These detailed connectivity data will provide new insights into the information transmitted through these IC subdivisions and guide future research to understand the functional roles of these pathways.

SU173. Effects of Fluid Volume, Viscosity, Head Orientation, and Negative Pressure on Wideband Acoustic Immittance in Middle-Ear Effusion and Residual Fluid

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Background: Otitis media with effusion (OME) is among the most prevalent conditions in the pediatric population and represents the leading cause of conductive hearing loss in children. In neonates, residual amniotic or other postnatal fluid in the middle ear can also complicate newborn hearing screening, contributing to false-positive referrals. While fluid volume and head orientation have been recognized as important factors, the combined influence of negative middle-ear pressure and the wide range of effusion viscosities (spanning ~1–10,000× that of water, from serous to thick mucoid) on acoustic responses remains poorly characterized.

Methods: We extended a previously validated finite-element (FE) model of middle-ear wideband acoustic immittance (WAI), tuned using simulation-based inference. Parametric sweeps examined the effects of effusion volume, head orientation, negative pressure loading, and viscosity. Baseline simulations with an air-filled cavity provided reference immittance and absorbance spectra. Comparative analyses were performed against both our own and published clinical data.

Results: Simulations showed that fluid volume and head orientation strongly altered WAI through changes in effective air space and variations of fluid–TM contact area, producing spectral shifts consistent with clinical observations. Negative pressure further accentuated impedance changes, particularly at low frequencies. Increasing viscosity reshaped the absorbance profile, introducing sharper peaks shifted above 1 kHz.

Conclusions: This study demonstrates how a fluid–structure interaction framework can explain the combined influences of testing conditions (e.g., head orientation) and pathological changes (e.g., fluid presence, volume, viscosity, and negative pressure) on WAI. By capturing these diverse conditions, the model provides mechanistic insight into how OME alters middle-ear transmission and how variability in measurement settings can shape observed WAI responses. Because WAI reflects the acoustic transmission properties of the ear, these findings also help to

interpret how middle-ear status influences otoacoustic emissions (OAEs, forward and backward transmission) and auditory brainstem responses (ABRs, forward transmission). The effect of head orientation is particularly relevant for newborns, who are typically tested in convenient, non-standardized resting positions that may increase variability, reduce test–retest reliability, and contribute to false-positive referrals. Finally, the framework establishes a foundation for applying machine-learning approaches trained on diverse fluid and orientation scenarios to improve diagnostic accuracy in pediatric OME evaluation.

SU174. Sensitivity-Guided Inference: A Structured Approach to Reduce Simulation Burden in Neural Network–Based Diagnosis

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Background: Neural networks and deep learning are increasingly used for diagnostic modeling, but their growing complexity often overshadows traditional statistical analyses essential for interpretability. Simulation-based inference (SBI) learns the mapping between model outputs and parameters and, given reference data, returns posterior distributions that can be fed into a deterministic model to reproduce the data. However, as the number of parameters increases, required simulations grow rapidly (approximately quadratically with dimensionality), slowing convergence and limiting interpretability. The middle ear, with its complex structure and many parameters, is a prime case: we propose a two-stage strategy that first ranks parameter influence via a global sensitivity procedure and then applies tiered SBI to sequentially tune high-dimensional models (from high- to lower-sensitivity subsets). This enables automated diagnosis and can inform middle-ear reconstructive surgery planning.

Methods: We propose a structured workflow that integrates systematic sensitivity analysis with multi-batch SBI to reduce simulation burden while retaining interpretability. As a case study, we implemented our approach on a lumped-element human middle-ear model that has been experimentally validated. Parameters were first ranked using one-at-a-time sweeps and global methods (Morris and Sobol total-order indices) to identify and order model parameters by their influence on system outcomes. Parameters were grouped into high-, mid-, and low-impact tiers. Multi-batch SBI was then performed iteratively: first inferring high-impact parameters while fixing others at prior baselines, then sequentially unfreezing mid- and low-impact groups. This strategy reduces dimensionality at each stage while updating priors with maximum a posteriori (MAP) estimates.

Results: Sobol ranking with Saltelli’s sampling scheme ($\approx 30,000$ simulations) produced a clear hierarchy of influential parameters. Learning-curve analysis guided the number of simulations needed for each tier, and staged multi-batch SBI yielded stable posteriors and reproducible spectra. In contrast, conventional all-parameter SBI required orders of magnitude more simulations and often failed to converge consistently or reproduce reference experimental data.

Conclusions: Sensitivity-guided, tiered inference reduces the simulation burden of SBI while preserving interpretability. The framework provides a practical workflow for parameter ranking, staged inference, and efficient posterior convergence. Applied here to the middle ear as a case study, the approach demonstrates how complex biological systems with many parameters can be analyzed in a way that identifies the properties most critical to system function while ensuring

that neural network–based diagnosis remains computationally efficient and clinically interpretable rather than a black box.

SU175. A Unified Framework for Spectral Similarity and Parameter Confidence in Neural Network–Based Diagnosis

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Background: Simulation-based inference (SBI) uses neural networks to map model parameters to outputs. This overcomes limitations of deterministic models that rely on fixed parameter sets, as SBI can capture uncertainty and variability. However, two challenges remain: (1) quantifying how well SBI-informed outputs match experimental spectra and (2) assessing confidence in inferred parameters. We propose a general framework that addresses both aspects, demonstrated here using middle-ear wideband immittance spectra.

Methods: Experimental reference data included repeated measurements of stapes velocity, ear-canal impedance, and absorbance (200 Hz–8 kHz) on a temporal bone. A finite-element model was used to generate training datasets with randomized parameter values, and SBI was trained to infer posterior distributions of seven physiologically meaningful middle-ear parameters. Spectral similarity was evaluated using three categories of measures:

- Point-to-point error metrics: MAE, RMSE, weighted RMSE, L1 and L2 norms, and maximum absolute error
- Distributional/divergence metrics: Wasserstein distance, AUC difference, and energy distance.
- Shape/trend metrics: dynamic time warping, cosine similarity, Pearson correlation coefficients.

Posterior confidence was quantified using entropy and distributional width measures (standard deviation, interquartile range (IQR), full width at half maximum (FWHM), mass width). Learning-curve analysis, varying simulation dataset size from hundreds to 10,000, identified the minimum data required for stable convergence of SBI training. Noise-sensitivity analysis evaluated the robustness and narrowness of posterior distributions under experimental variability. A majority-vote scheme integrated all metrics into two composite indices: the Spectral Similarity Index (SSI) and the Parameter Confidence Index (PCI).

Results: The SSI and PCI jointly identified conditions where SBI produced both accurate spectral fits and narrow, well-constrained parameter distributions. Learning-curve analysis revealed diminishing returns beyond a critical dataset size, while noise analysis exposed stability limits of posterior estimates. Importantly, SSI and PCI were calculated simultaneously across stapes velocity, ear-canal impedance, and absorbance, yielding a joint composite index rather than independent similarity scores for each spectrum.

Conclusions: This framework moves beyond single-metric evaluation by combining multiple similarity and confidence measures into unified indices. Although demonstrated here for model-to-data comparisons, it can be generalized to contexts where multiple spectral datasets (e.g.,

data-to-data) are compared. In doing so, it supports more robust model validation and strengthens confidence in parameter-based clinical decision-making.

SU176. Acoustic Measurement of Tympanic Membrane Displacement During Middle Ear Muscle Reflex Activation in Normal Human Ears

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Background: The same acoustic stimulus paradigm that has been used to detect middle-ear muscle reflex (MEMR) may be further analyzed to estimate the distance between the ear-canal probe and the tympanic membrane (TM). This study examines TM displacement in response to MEMR activation to infer contraction of the stapedius and/or tensor tympani muscles.

Methods: A train of acoustic clicks presented to the ear canal served as probes, and a contralateral broadband sound that increased and decreased in sound level served as a putative MEMR elicitor. Analysis of the acoustic response to click stimuli can provide meaningful interpretation when both magnitude and phase are used. Further analysis of the click response using the MEZ(Trademark) method (Merchant and Neely, 2021) provides estimates of middle-ear input impedance and TM displacement. An analog-circuit model of the ear canal and middle-ear was fit to individual click responses. The time-course of model components associated with the ossicular chain suggests how its stiffness and damping are altered by MEMR activation. The direction of TM displacement may indicate whether MEMR activation is dominated by contraction of the tensor tympani (medial) or stapedius (lateral) muscle.

Results: In the subset of measurements where (1) ear-canal impedance and middle-ear impedances were well fit by the model and (2) TM returned to its initial displacement after termination of the contralateral elicitor, TM displacement was about equally likely to be medial or lateral. Distributions of model component values across all well-fit measurements showed a significant decrease ($p < 0.01$) in stiffness of the ossicular chain and no significant change in other components.

Conclusions: Our findings are supported by a recent study of human temporal bones (Puria, 2023), which observed that contraction of the tensor tympani muscle or the stapedius muscle laterally displaces the umbo of the malleus either laterally or medially, respectively. Our approach may provide an avenue to understand the function of the tensor tympani muscle, which has remained poorly understood.

SU177. Leveraging Middle-Ear Input Impedance for Diagnosis of Conductive Pathologies

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Background: Machine learning-based classification has the potential to improve the accuracy and efficiency of diagnosing mechanical ear pathologies when applied to audiometric and wideband tympanometry (WBT) data. Previous studies show that pairing the air-bone gap (ABG) with absorbance, the most widely used WBT metric, yields better discrimination between stapes fixation (SF) and superior canal dehiscence (SCD) than either measure on its own. However, absorbance captures only magnitude and omits phase information, limiting its ability to fully characterize middle- and inner-ear mechanics. Impedance offers an advantage in this respect, but variability in ear canal acoustics may obscure its diagnostic value. By using a circuit model of the ear, these ear canal effects can be minimized, allowing the input impedance at the middle ear (ZME) to be estimated.

Methods: 97 ears were included in this study: 27 normal, 32 with confirmed SCD via CT scan, and 38 with SF confirmed via surgery. WBT data were collected with the Interacoustics Titan, and air- and bone-conduction thresholds (0.25-4 kHz) were obtained from patient audiograms. ZME was derived for each ear by fitting a lumped-element model composed of three parallel branches, each containing a stiffness (K), damping (R), and mass (M) element. The ear canal was represented as a transmission line to isolate ZME from the measured input impedance at the ear canal (ZEC). Logistic regression algorithms were trained with dimensionality reduction via PCA, and cross-validation was used to evaluate classification performance.

Results: The combination of ABGs and |ZME| achieved the lowest classification error rate (15%), outperforming ABGs and absorbance (22%). ABGs alone resulted in 20% error. Confusion matrix analyses showed that SF could be classified with perfect accuracy (100%) using ABGs and |ZME|, but SCD remained more difficult to identify, with correct rates under 70% across algorithms.

Conclusions: These findings suggest that impedance, when paired with ABGs, improves diagnostic classification compared to absorbance. Such classification algorithms could reduce reliance on imaging or surgical exploration, particularly for differentiating SF from SCD. Future work includes expanding the dataset, incorporating other conductive pathologies (e.g., ossicular discontinuity, malleus fixation), and exploring alternative model representations. In addition, the role of impedance across static pressure sweeps remains poorly understood. Future work also includes investigating whether the full pressure range from -300 daPa to +200 daPa may serve as an additional tool for distinguishing pathologies.

SU178. High-Frequency Bone-Conduction Thresholds (4–16 kHz) in Children: Feasibility, Reliability, and Early Disease Signatures

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Background: Bone-conduction (BC) hearing thresholds are rarely measured above 4 kHz in routine practice, despite well-documented examples of high-frequency (HF) conductive hearing loss. Newly characterized BC transducers (Tascam and KLH96) with high output and low distortion permit reliable HF-BC measurement (4–16 kHz) in adults. We implemented HF-BC in

pediatric otology clinic to (i) assess thresholds in normal-hearing (NH) pediatric ears and (ii) explore HF hearing loss signatures of common middle ear disease in pediatric patients.

Methods: Children 5–16 y underwent standard and HF audiometry including air-conduction (AC) and BC thresholds at 4, 6, 8, 9, 10, 11.2, 12.5, 14, and 16 kHz. Contralateral masking was used when HF-AC asymmetry was ≥ 10 dB. Reliability was tested via serial threshold measurement at select frequencies (9 and 12.5 kHz). Audiometer output was converted to force (dB re 1 μ N) and to hearing level (dB HL) using our published HF-BC calibration protocol and adult reference thresholds (18–25 y). Pediatric cohorts included NH, otitis media with effusion (OME; flat tympanogram), and post-middle-ear surgery/disease (“post-surgical”).

Results: HF-BC thresholds were feasible as early as age 5 and measurable at 4–16 kHz. Reliability was typically ≤ 5 dB at 9 and 12.5 kHz. Pediatric median force thresholds were broadly similar to adult NH subjects; but select frequencies (4, 8, 16 kHz) showed pediatric medians ~ 10 –15 dB lower (better). Thresholds obtained with the KLH96 and Tascam in the same subjects were generally similar to test-retest variability with larger differences at 4–6 kHz, indicating a possible need for device-specific reference values.

Disease signatures: In an OME case followed longitudinally, HF-AC improved after tympanostomy tube placement while HF-BC remained stable, consistent with an HF air-bone gap (ABG) due to HF conductive hearing loss. In other post-surgical ears, we observed two patterns: (i) a conductive signature (stable HF-BC with improved HF-AC), and (ii) a sensorineural signature (HF-BC thresholds elevated relative to pediatric NH medians and tracking with AC). These examples illustrate HF-BC’s ability to separate conductive from sensorineural contributions at high frequencies.

Conclusions: HF-BC audiometry (4–16 kHz) is feasible in children, reliable at 8–12.5 kHz, and clinically informative for distinguishing type of HF loss in common pediatric conditions. Pediatric force medians are largely similar to adults but may be lower by ~ 10 –15 dB at select bands; larger datasets are needed to determine whether pediatric-specific RETVFLs are warranted. Findings also motivate parallel investigations in HF-AC references in this population. Overall, HF-BC provides a practical path to quantify HF ABGs and separate conductive from sensorineural effects to guide counseling and improve surgical outcomes.

SU179. Avian Cochlear Nucleus Neurons Exhibit a Tonotopic Developmental Delay of Hcn Current

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Background: Functional hearing relies on the precise frequency-specific encoding of auditory stimuli. Nuclei in the auditory brainstem, such as the mammalian cochlear nucleus and the analogous avian nucleus magnocellularis (NM), are organized by different frequency-specific regions known as tonotopy. HCN channels across different frequency regions help support tonotopic tuning by the underlying mediated current known as I_h . While it is known that low-frequency regions of NM exhibit a developmental delay compared to the high-frequency areas,

differences in HCN channel expression and I_h across development and tonotopic regions remain unknown. This study aims to fill this knowledge gap.

Methods: We recorded from high- and low-frequency regions (HF and LF, respectively) of brainstem slices before (embryonic (E) days 12-13), during (E15-16), and after hearing onset (E20-21). Voltage clamp recordings from NM neurons measured I_h from holding voltages between -40mV to -130mV in 10mV increments. I_h was measured with Steady State (SS) and different current techniques and compared. Fast and slow I_h activation kinetics were fit with single and double exponentials to explore HCN channel subunit contribution.

Results: I_h increases significantly across development for all NM neurons. However, in embryos before hearing onset, high-frequency neurons have significantly more I_h than low-frequency neurons. In embryos during hearing onset, HF and LF difference currents are similar, while HF neurons still have a greater inward SS current than LF neurons. Finally, for embryos after hearing onset, there is no significant difference between HF and LF neurons for either SS or difference current. Activation kinetics are similar across the tonotopic axis throughout development.

Conclusions: Results suggest that at the beginning of neural circuit development, HF neurons have more HCN channels than LF neurons. Furthermore, I_h magnitudes increase across development with HF neurons developing sooner than LF neurons with respect to HCN channels. Since activation kinetics do not differ across development or tonotopy, results suggest that subunit composition of HCN channels is not changing during development, nor is it different across the tonotopic axis. Taken together, we conclude that LF neurons experience a developmental delay in the number of HCN channels present, with I_h catching up to HF neurons by the onset of hearing. Characterizing the development of auditory brainstem neurons across the tonotopic axis is a crucial step in elucidating how normal central auditory systems function effectively in vertebrates.

SU180. Auditory-Visual Speech Integration is Supported by Frontal-Cortical Engagement in Older Adults

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Background: Older adults with age-related auditory (AO) and visual (VO) speech perception deficits can often identify audiovisual (AV) speech to a degree of accuracy like that of younger adults. We have previously reported that this enhanced AV speech perception (relative to unisensory deficits) by older adults can be explained by auditory-visual multisensory perceptual benefits that are > in younger adults. Poorly understood, however, are the neural mechanisms that support AV speech perception in older adults with age-related auditory and visual deficits. Cortical activity in inferior-frontal and temporoparietal cortex has previously been found to be a stronger predictor of AV speech encoding in older adults than younger adults, especially in noisy listening conditions, suggesting that higher-level cortical engagement may support auditory-visual speech integration in older adults. Here we examined how the functional connectivity between inferior-frontal cortex and temporoparietal cortex can predict auditory-visual enhancement of AV speech perception in younger and older adults. We hypothesized that

stronger functional connectivity between cortical areas would predict more auditory-visual enhancement in older adults than in younger.

Methods: A group of 10 younger normal-hearing adults (20-30 years of age, 7 female) and 14 older adults (54-80 years of age, 7 female) with normal hearing or mild-to-moderate sensorineural hearing loss completed an audiovisual speech identification task, identifying words spoken in Gaussian noise (-5, 0, +5 dB SNR) presented in AO, VO, and AV conditions. We calculated auditory-visual enhancement from identification performance across conditions [AVE=AV-(AO+VO)]. Speech-evoked potentials (SEPs) were recorded separately while participants were passively presented consonant-vowel syllables (/ba/, /da/, /ga/) in audiovisual conditions in silence and in Gaussian noise at 0 dB SNR (240 trials per condition). Each participant's AV SEP and T1 structural image were used to source-constrain their SEPs to inferior-frontal cortex and temporoparietal cortex to measure the response generated in each cortical area. For each participant, metrics of functional connectivity between inferior-frontal and temporoparietal cortex were computed for responses evoked by AV speech (cross-correlations and dynamic time warping distance).

Results: AV SEP response latencies in inferior-frontal and temporoparietal cortex were longer for older adults than younger adults in inferior-frontal and temporoparietal cortex. AV response latencies in inferior-frontal cortex were shorter in noisy listening conditions, but only for older adults. Importantly, greater functional connectivity between inferior-frontal and temporoparietal cortex when listening to AV speech in noise predicted larger multisensory benefits in speech identification (AVE).

Conclusions: These preliminary findings suggest that older adults rely on a functional network between inferior-frontal and temporoparietal cortex when identifying AV speech in noisy listening conditions, more so than younger adults. The results will be discussed with regards to age-related changes in sensory processing and cognition and the potential role of visual input in alleviating age-related deficits in auditory speech communication.

SU181. Mechanisms for Generation and Control of EMREOs (Eye Movement-Related Eardrum Oscillations)

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Background: Accurate information about eye movements is necessary for audiovisual integration because such movements shift the positions of the eyes with respect to the head/ears. We have recently reported an eardrum oscillation, time-locked with eye movement onset and occurring in the absence of outside auditory stimuli, that suggests eye movement information is sent by the brain to the ears (Eye movement-related eardrum oscillation, EMREO; Gruters et al. PNAS 2018; Lovich et al. PNAS 2023). But what is the proximal and neural mechanism producing such eardrum oscillations? The chief suspects are the middle ear muscles (MEMs) and the outer hair cells (OHCs).

Methods: We recorded eye movements and associated eardrum oscillations in rhesus monkeys before and after surgical intervention involving the MEMs -- specifically, stapedotomy and

tensor tympani tenotomy. Such transections of the tendons connecting the MEMs to the ossicles are typically done in human patients suffering from otosclerosis or MEM myoclonus, and the procedures were adapted here for the monkey. The procedures served to eliminate the contribution of the particular MEM in question to the EMREO.

Results: Sectioning MEM tendons did not eliminate the EMREO. Following stapedotomy, the EMREO was reduced in amplitude but still occurred. Following tensor tympani tenotomy, the EMREO was actually increased in amplitude. When stapedotomy and tensor tympani tenotomy were combined, the EMREO still occurred, and was again increased in amplitude. Together these findings suggest the MEMs are not the sole cause of the EMREO, but rather regulate its amplitude likely in a push-pull fashion. These results from animals are largely consistent with related preliminary data in human clinical patients who have undergone similar procedures.

A general caveat to the interpretation of the MEM surgical interventions is that the procedure may change the mechanical load on the eardrum such that interpretations of changes in amplitude of the EMREO signal should be confirmed by additional research. However, that the EMREO continued to exist even after both MEMs were disconnected from the ossicles indicates that there must be an additional source of force contributing to the signal. The most likely such source is the OHC, which could potentially generate this signal by oscillating in concert throughout the cochlea. It is also possible that in the event of OHC dysfunction, the signal can be generated by MEMs acting alone, a possibility not tested here.

Conclusions: We interpret these findings in the context of how eye movements may alter sound localization cues. Recent work has shown that forces applied by the MEMs to the eardrum in a cadaver model could alter both interaural timing and level difference cues in an eye movement-dependent way (Cho et al., Hearing Research 2023).

SU182. Component Analysis of Distortion Product Otoacoustic Emissions in Children for f2 up to 12 kHz

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Background: Distortion product otoacoustic emissions (DPOAE) recorded in the ear canal are mixtures of non-linear distortion and linear reflection components. Each component has its own hypothesized generation source and mechanism based on its phase-frequency behavior. However, little is known about the components' behavior in the first decade of life particularly for $f_2 > 8$ kHz. Here we report on individual DPOAE components measured using low stimulus levels, which are more sensitive to cochlear status yet difficult to measure in noisy ears. Thus, multiple signal cleaning strategies for handling noisy recordings are described.

Methods: 94 children between the age of 6 weeks and 11 years were recruited from a maternity hospital in São Paulo, Brasil. With the Etymotic Research 10X system, DPOAE were measured using fixed ratio of 1.22 for f_2 frequencies between 1.5 and 12 kHz and $L1/L2 = 55/40$ dB FPL using a 96 kHz sampling rate. Time domain waveforms were sent through a multi-step artifact rejection protocol followed by a least-squares-fit based frequency analysis and weighted

averaging procedure. The complex level and phase were then processed using continuous wavelet transform, during which the complex signals were convolved with an array of mother wavelet basis functions and resulting coefficients were filtered into different latency bands according to Moleti et al (2008). The filtered signals were separated as zero-latency distortion components and non-zero-latency reflection components. The time-frequency representations as well as level and phase behaviors were compared for three signal cleaning methods.

Results: 76 ears had recordable DPOAE above the noise floor at primary levels of 55/40 dB FPL. Nine ears with the highest quality recordings as determined by 9 dB SNR criteria across 90% of the spectrum were used as the gold standard for our analysis. The time-frequency representations of these nine ears demonstrated DPOAE energy hotspots that were constrained in the first and second latency bands with little to no signal outside of these bands. In contrast to these nine exemplar recordings, the time-frequency representations of noisier recordings displayed energy at latencies outside of the expected frequency-latency function at randomly distributed spectral regions. Furthermore, phase behavior of the total DPOAE and distortion component occasionally varied with frequency, a phenomenon not consistent with the fixed phase of distortion emissions in fixed-ratio recordings.

Conclusions: The influence of physiological and environmental noise on DPOAEs evoked by low stimulus levels must be considered when separating individual components. Advanced signal processing methods such as SNR-weighted averaging, multi-step artifact rejection, continuous wavelet transform that can numerically quantify the unwanted noise residuals, and time-frequency representations that can visually display noise contributions may be useful and will be discussed. Future studies are warranted to examine the utility of these methods for improving detection of DPOAE responses at low stimulus levels.

SU183. A Transmission Line Model of the Cochlea Under Noise Produces Four Distinct Categories of Spontaneous Emissions

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Background: Spontaneous Otoacoustic Emissions (SOAEs) are indicative that cochlea is an active, nonlinear system. Existing research has shown the transmission line models of the cochlea with roughness can predict narrow band peaks in the absence of any external stimulus, i.e. SOAEs; however, these models are limited to only describing narrowband bimodal peaks. Existing human SOAE experimental data show that SOAEs include not only narrowband bimodal peaks but also broadband unimodal peaks.

Methods: The cochlea is studied using a modified version of the Ku and Elliot model, which is a 1D transmission line model. Roughness is introduced by adding small (0.25%) perturbations to the activity parameter γ . Full spectrum white noise is applied to the basilar membrane and is uncorrelated in time and space. The nonlinear dynamics of the model are simulated numerically in the time-domain before analyzing the spectrum of the response at the stapes and evaluating how the key characteristics of the spectrum are linked to the linear stability analysis of the model. Spectral peaks are characterized by their quality factor (tuning sharpness), level, and their time domain distribution.

Results: The model with noise predicts a series of spectral peaks with a wide range of levels, quality factors, and Hartigan dip statistic values. Comparison of the SOAE spectrum to the linear stability diagram (eigenvalues of the state-space matrix) helps to interpret the numerical results. Our analysis suggests that SOAE peaks can be naturally divided into four categories: (1) Primary SOAE Type A peaks; (2) Primary SOAE Type B peaks; (3) Filtered noise peaks; (4) and Distortion product SOAE (DPSOAE) peaks. Primary SOAE Type A and B peaks are associated with unstable eigenvalues; Type A appear in the noiseless case and the case with noise, and Type B appear only in the case with noise. Filtered noise peaks are associated with nearly unstable eigenvalues and act as lightly damped harmonic oscillators. Distortion product SOAE peaks are peaks which are distortion products of primary SOAE peaks.

Conclusions: With an extension to the existing linear stability analysis and the introduction of noise into the transmission line model of the cochlea, we demonstrate how the shortcomings of existing transmission line models can be overcome, and we observe additional phenomena from the introduction of noise which was not previously predicted. The presence of different categories of SOAE peaks provides a novel lens through which to understand SOAE spectra through.

SU184. Growth of DPOAEs Evoked by the Fixed L1 Stimulus Paradigm

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Background: Distortion product otoacoustic emission (DPOAE) magnitudes measured across a range of stimulus levels as level growth functions (LGFs) may be useful for assessing cochlear function and health. The observed LGF shapes depend on the variety of stimulus paradigms that have been reported in the literature. Recent work has suggested that the slopes of LGFs from the fixed L1 paradigm may differentiate types of cochlear hearing loss; however, normal LGF growth in this paradigm is not well understood. LGFs are typically fitted with mathematical functions from which slopes can be derived and (behavioral) thresholds can be predicted. However, fitting LGFs at low stimulus levels is challenging, due to poor signal-to-noise ratios (SNRs). Here, we describe a generalized linear statistical model (GLM) appropriate for fitting LGFs at varying SNRs, including portions of the LGF that fall below the noise floor. The primary aim of this study was to characterize fixed L1 LGFs at low-to-moderate levels in young listeners. A secondary aim was to evaluate the hypothesis that functions fit to these LGFs accurately predict DPOAE magnitude at low stimulus levels ($L2 = 5$ dB FPL), which produce responses that are typically below measured noise floors.

Methods: DPOAE LGFs were recorded from 12 young, healthy adult participants (9 female, mean = 22.9 yr) using an ER10X probe system and custom software. L1 was held constant at 65 dB FPL, while L2 swept in level from 0 to 70 dB FPL in 9 seconds. Each test consisted of 24 nine-second sweeps for a total test time of 216 seconds. For evaluating the fit at low levels, discrete measurement was also obtained at $L2 = 5$ dB FPL, a low stimulus level eliciting DPOAEs well below the typical noise floor. This low-level DPOAE was recorded for a long period of time (~26 minutes averaging time) to reduce the noise floor to near -40 dB SPL. A phenomenological model was used to generate simulated data for comparison with human data.

Results: Data from healthy young adult listeners, along with simulations from a phenomenological model, established that at low to moderate L2 and a fixed L1, LGFs grow as polynomials dominated by the linear term, with smaller but important contributions from the quadratic and cubic terms. The GLM predicted DPOAE magnitudes at low stimulus levels with a mean error < 0.4 dB (SD = 2.2 dB).

Conclusions: This improved understanding of expected LGFs in healthy ears provides a foundation for future studies of the relationship between fixed L1 LGFs and cochlear health.

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SU185. Auditory Novelty Detection Impairment in Rats Treated With the Nmda Receptor Antagonist MK-801: A Model of Schizophrenia

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Background: Animals are constantly exposed to a wide range of environmental sounds. While many of these are irrelevant, the detection of novel stimuli is crucial for survival. Behavioral habituation, a fundamental form of learning, is characterized by the progressive reduction of responses to repeated stimuli and provides the foundation for detecting unexpected changes (Rakin, 2009; Netser et al., 2011; Quintela-Vega et al., 2023). This ability is closely linked to mismatch responses observed at both neuronal and behavioral levels, which have been conceptualized within the predictive coding framework. These responses rely on repetition suppression to predictable stimuli and prediction error signals when expectations are violated. Importantly, reduced mismatch responses have been consistently associated with schizophrenia and glutamatergic dysfunction in humans. To investigate this, we used a pharmacological model based on the NMDA receptor antagonist MK-801, which reproduces schizophrenia-like features by disrupting neuronal adaptation and associative learning mechanisms (Parras et al., 2017; Ishishita et al., 2019).

Methods: We trained eight freely moving female Long Evans rats to discriminate deviant tones in an oddball paradigm. Animals learned to nose-poke in response to deviants, which were rewarded with food pellets. A pair of frequencies within the auditory range (8.0–11.3 kHz) was used, and discrimination was tested across inter-stimulus intervals (1.0, 1.5, and 2.0 s) with a 10% deviant probability. A many-deviant paradigm was also applied to control for predictive coding indices: mismatch (iMM), prediction error (iPE), and repetition suppression (iRS). Behavioral performance was quantified using d' index. Once the animals reached reliable performance (d' > 1), they were divided into two groups: control and MK-801. Both saline and MK-801 (0.075 mg/kg) were administered subcutaneously 45 min before the behavioral deviance detection task. Finally, neuronal activity was recorded in the auditory cortex and the inferior colliculus using multi-channel probes and equivalent paradigms to study neuronal responses.

Results: Preliminary results show that saline-treated animals continued to improve in the deviance detection task during treatment, whereas MK-801-treated rats exhibited a significant impairment in performance after drug administration.

Conclusions: These findings suggest that NMDA receptor blockade with MK-801 disrupts auditory novelty detection at both behavioral and neural levels. This pharmacological model provides valuable insights into the mechanisms underlying sensory and cognitive deficits in schizophrenia.

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SU186. Serotonergic Modulation of Auditory Cortical Circuits

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Background: Auditory experience powerfully shapes the functional organization of the developing auditory cortex (ACtx), but this plasticity declines in adulthood. Neuromodulator release can reinstate robust plasticity in the adult brain, offering potential to promote recovery from neurological disorders or peripheral hearing loss. However, the mechanisms by which distinct neuromodulators act within cortical circuits to drive plasticity remain incompletely understood. For example, serotonin (5-HT) modulates tonotopic plasticity in the adult ACtx, but the underlying cellular and circuit mechanisms are unknown. Prior studies, including our own, have mapped serotonergic axons throughout ACtx and localized diverse 5-HT receptor mRNAs to distinct populations of excitatory pyramidal neurons and GABAergic interneurons. Specifically, subsets of deep-layer somatostatin-expressing (SST) inhibitory interneurons predominantly express transcripts encoding inhibitory 5HT1A receptors and may therefore serve as key targets of serotonergic modulation. We hypothesize that 5-HT selectively suppresses SST inhibitory interneurons to dynamically sculpt network activity.

Methods: To identify the effects of 5-HT on SST interneurons, we combined whole-cell electrophysiology and two-photon (2-P) imaging in thalamocortical slices of mouse ACtx.

Results: Local 5-HT application reliably hyperpolarized layer 5 (L5) SST interneurons and suppressed spike amplitude, which was abolished by bath application of a selective 5-HT1A antagonist. Given that subsets of SST interneurons expressing the 5-HT1A receptor form local projections, we employed optogenetic activation of SST interneurons to probe serotonergic modulation of SST inhibitory output onto L5 pyramidal neurons. We observed that bath-applied 5-HT reduced SST-mediated inhibition onto the majority of these neurons.

SST interneurons have been previously shown to curtail runaway excitation during periods of prolonged cortical activity. Using 2-P imaging to interrogate the effects of 5-HT on the cortical network, we demonstrated that 5-HT prolongs calcium responses evoked by repetitive thalamic

stimulation in subsets of L5 pyramidal neurons, consistent with reduced SST-mediated inhibition.

Conclusions: Ongoing work is examining whether serotonergic actions on specific SST interneuron subclasses mediate layer-specific modulation. L5 serves as the principal output layer of the cortex, suggesting that 5-HT may gate information flow from primary ACtx to higher-order cortical and subcortical targets. Together, these studies provide new insight into the cellular and circuit mechanisms by which 5-HT modulates ACtx, informing the development of targeted strategies to induce layer-specific neuronal changes that may enhance adult cortical plasticity.

SU187. Decision-Making Shapes Auditory Cortical Responses to Harmonic Complex Tones in Noise

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Background: Background noise often presents a challenging acoustic environment for listening. Adaptive plasticity in auditory cortex is thought to facilitate hearing in noise by adjusting the gain of neural responses to behaviorally meaningful sounds. However, it remains unclear how adaptive plasticity facilitates the recognition of auditory perceptual features with masked acoustic components, such as the missing fundamental frequency (F0) of a harmonic complex tone (HCT) in background noise. Since mice have high-frequency hearing, neurons in auditory cortex that are tuned to low F0s encode missing-F0s in HCTs. Thus, we hypothesized that adaptive plasticity in mouse auditory cortex facilitates the recognition of missing-F0s in noise by modulating the activity of F0-selective neurons relative to background noise level.

Methods: Background noise often presents a challenging acoustic environment for listening. Adaptive plasticity in auditory cortex is thought to facilitate hearing in noise by adjusting the gain of neural responses to behaviorally meaningful sounds. However, it remains unclear how adaptive plasticity facilitates the recognition of auditory perceptual features with masked acoustic components, such as the missing fundamental frequency (F0) of a harmonic complex tone (HCT) in background noise. Since mice have high-frequency hearing, neurons in auditory cortex that are tuned to low F0s encode missing-F0s in HCTs. Thus, we hypothesized that adaptive plasticity in mouse auditory cortex facilitates the recognition of missing-F0s in noise by modulating the activity of F0-selective neurons relative to background noise level.

Results: Mice successfully discriminated between target vs. non-target F0s in silence ($d' > 1$), with performance declining as SNR decreased. Average neural responses to HCTs also declined with SNR, however, neural responses depended strongly on behavioral choice. As SNR decreased, neural responses during behavioral hits and false alarms increased and decreased, respectively. In contrast, neural responses during correct rejections and misses were less choice-dependent, generally increasing with noise level.

Conclusions: Our results suggest that during perceptual discrimination of missing-F0s, neural responses to HCTs in noise reflect a combination of sensory coding and decision-making processes in auditory cortex. Together, these mechanisms may facilitate listening in challenging acoustic environments.

SU188. Maturational Changes in Listening Effort During a Degraded Speech Task in Adolescents and Young Adults

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Background: Understanding speech in challenging listening conditions, such as noisy environments or with degraded signals, is a skill that requires intact auditory encoding, deployment of listening effort, and application of cognitive skills. While some studies have indicated that perception of degraded speech continues to mature during adolescence, others have not. Here we examined (1) whether behavioral performance on a degraded speech task changes during adolescence and young adulthood, (2) how adolescents and young adults deploy listening effort during this task, and (3) the relationship between task performance and listening effort over this age range.

Methods: We tested 10- to 23-year-olds (n=76) on 6-band noise-vocoded (NV) speech under two conditions: NV speech immediately followed by a period of silence or NV speech immediately followed by multi-talker babble. Listeners were asked to repeat back what they heard following either the silent period or the babble. Based on Winn and Moore's (2018) prior work with hearing-impaired listeners, we hypothesized that it would require more listening effort for all listeners to comprehend the speech when it was followed by babble, but that these effects would be more pronounced in adolescents. We assessed listening effort using an eyetracker to measure pupil size during pre-trial baseline, the sentence, the babble or silence, and the participant's response (i.e., repeating back the sentence). We analyzed pupil size change relative to baseline over the course of the trial and compared these temporal dynamics between the silence and babble conditions and between the age groups. Consistent with convention in the field, larger pupil size compared to baseline was interpreted as indicative of greater listening effort.

Results: The temporal dynamics of listening effort differed between the age groups and the two listening conditions. In addition, the adolescents demonstrated greater listening effort despite poorer performance on the degraded speech comprehension task.

Conclusions: These data suggest that degraded speech perception continues to mature during adolescence. Poorer performance by teenagers occurred despite higher levels of listening effort, indicating that their prolonged perceptual development is not attributable to poor engagement. Other factors that may contribute to this late maturation include developmental changes in auditory encoding and cognitive processing.

SU189. Ideal Observer Analysis of the Effects of Medial Olivocochlear Gain Control on Neural Coding of Sound Level and Amplitude Modulation

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Background: The medial olivocochlear (MOC) system reduces cochlear gain in response to sound. One hypothesis for the MOC system's role is that it adjusts cochlear gain to improve

sound coding at moderate sound levels. For example, when detecting amplitude modulation (AM) of a mid-level signal, a reduction in cochlear gain could reduce the deleterious effects of cochlear compression and/or synaptic saturation and enhance AM detection. This gain reduction could be elicited by sound preceding the AM stimulus (precursor). Some psychophysical data on level discrimination and AM detection with precursors appear qualitatively consistent with this hypothesized role of the MOC system. However, due to nonlinearities present in the auditory periphery, it is difficult to predict how much cochlear gain changes should be expected to change behavioral thresholds.

Methods: To quantitatively predict the effects of MOC gain control on level discrimination and AM detection, we used a model of the auditory periphery and midbrain modified to include a simple MOC reflex driven by stimulus energy. We simulated responses in this model to level discrimination and AM detection stimuli with and without precursors and then used ideal observer analysis to determine theoretical bounds on the effects of cochlear gain changes on task performance. Both rate-based and temporal coding schemes were evaluated, and we applied the framework to several stages in the model to better understand how the effects of MOC gain control manifest along the ascending auditory pathway.

Results: Ideal observer analysis revealed that, at moderate sound levels in single model channels, reductions in cochlear gain enhance level discrimination and AM detection, consistent with classical theories of MOC gain control. However, effect sizes for level discrimination and AM detection were small in peripheral model stages for both rate-based and temporal codes. For example, at 60 dB SPL in low-spontaneous-rate auditory-nerve fibers, a 10-dB change in cochlear gain resulted in a predicted change in level discrimination thresholds of only ~1 dB and AM detection thresholds of only ~2 dB, raising questions about whether these effects would be detectable behaviorally. Moreover, in contrast to single-channel results, when observers had access to off-frequency channels, predicted effects of cochlear gain on perceptual sensitivity were negligible.

Conclusions: Our simulations support the idea that the MOC system enhances coding of sound level and AM. However, at the same time, our simulations suggest that surprisingly large changes in cochlear gain may be required to produce measurable threshold changes in behavioral tasks designed to explore the MOC reflex.

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SU190. Measures of Subclinical Hearing Function: Long-Term Test-Retest Reliability and Relations Between Change Outcomes

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Background: Noise exposure is the main cause of preventable hearing loss (Rabinowitz, 2000) and recreational noise may pose a particular hazard in young people (WHO, 2015). Studies of hearing health in the younger population (e.g., Henderson et al., 2011; Carter, 2014; Keppler et al., 2015) have tended to focus on pure-tone audiometry (PTA: detection thresholds of pure tones

at different frequencies) but PTA may be relatively insensitive to the early effects of noise damage.

We conducted a longitudinal study investigating changes in auditory function over a three-year period, during which many participants started regularly attending high-sound-level environments (e.g., live music events and nightclubs). Our participants underwent an extensive battery of physiological, psychoacoustic and self-report measures with the aim of identifying early markers of noise damage. Here we report on relations between the various hearing change measures, with particular focus on disentangling the effects of hair-cell damage and synaptic pathology. We also report on the longer-term test-retest reliability of potential early markers of recreational noise damage in young people.

Methods: Participants were 220 teenagers aged 16 or 17 at the time of their baseline test session (Nov 2021 – Dec 2022). Follow-up test sessions took place from Nov 2024 to Jan 2026. At each test session, participants completed a battery of hearing tests:

- Cochlear function: Pure-tone audiometry (0.25-8 kHz), extended high-frequency audiometry (1/3-octave narrowband noise centred at 10 kHz, 12.7 kHz, and 16 kHz), and distortion-product otoacoustic emissions (2f1-f2 amplitude, at f2 frequencies of 3-16 kHz).
- Auditory neural function: Auditory brainstem responses to monaural filtered chirps and ipsilateral middle-ear muscle reflexes with two tonal probes (226 Hz and 1 kHz) and a 4-kHz tonal elicitor.
- Speech perception: Three digits-in-noise tests (monaural digits in speech-shaped noise, diotic digits in spatially separated babble, and diotic digits with two competing, concurrent, spatially separated digit streams).
- Self-report measures: short-lasting and long-lasting spontaneous tinnitus.

Hearing change measures were defined as the difference or the ratio between these outcomes measured at baseline and then again at follow-up (post – pre or post/pre).

Results: At the time of abstract submission, 180 of the 220 participants had been invited to complete the final testing session, of which 170 had attended. The study was powered based on 60% retention, yet participant retention was much higher: on track for ~89% (n = 195) by the close of the study. Data analysis was scheduled for the fourth quarter of 2025.

Conclusions: The large sample size, longitudinal design, and broad array of measures offer novel opportunities to explore the reliability and interrelations of subclinical hearing measures over the course of years. Results will have implications for the interpretation of research reliant on the use of such measures in young adults.

SU191. Identifying the Acoustic Fingerprints of Trigger Sounds and Predicting Discomfort for Misophonia

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Background: Human hearing is critical to everyday communication and the perception of natural auditory scenes. For individuals with misophonia, sounds commonly experienced in daily life can evoke severe discomfort and distress. Aversion is often described in terms of broad

sound categories, such as bodily sounds, but what acoustic features cause specific sounds to be aversive or not, within the same category or across different individuals, remains unclear. Here, we explore whether bottom-up statistical sound features processed in the auditory periphery and midbrain can explain aversion to sounds.

Methods: Using the Free Open-Access Misophonia Stimuli (FOAMS) dataset and a hierarchical model of the auditory system, we find that sound summary statistics extracted from the auditory model can predict discomfort ratings in participants with and without misophonia. The model was fit to each individual using Generalized Perceptual Regression (Clonan et al 2025) which produces individualized transfer functions that pinpoint specific spectrotemporal modulations that contribute towards sound aversion. Model parameters were also compared to diagnostic evaluations of misophonia (Duke Misophonia and S-Five questionnaires).

Results: Overall, the model explains 76% of the variance in discomfort ratings, and we find substantial differences across participants in which sound features drive aversion. An advantage of the approach here is that it is sound-computable – perceptual ratings can be fit from or predicted for any sound. To illustrate applications of sound-computable models, we consider 1) extrapolation to a large set of untested environmental sounds and 2) personalized trigger detection in continuous audio. Model predictions accurately predict aversion to FOAMS sounds and identify untested sound categories, not in the original FOAMS set, that may also be aversive. This suggests that there may be substantial heterogeneity in how aversive specific sounds are within some sound categories. In continuous audio, we show how sound-computable models can identify the timing of potential triggers from sound mixtures. Finally, parameters of the model that account for the listener’s perceptual sensitivity were significantly correlated with diagnostic evaluation scores.

Conclusions: Altogether, our results suggest that acoustic features – spectrotemporal modulations, in particular – can practically be used to characterize the individualized patterns of aversion in participants with misophonia. Future perceptual studies using synthetic sounds and sound sets with more diverse acoustics will allow model predictions to be tested more broadly; however, sound-computable models may already have applications in precision diagnosis and management of misophonia. Funded by NIH (R01DC020097).

SU192. The Contribution of Speech Articulatory and Statistical Sound Features Towards Speech Recognition in Natural Auditory Scenes

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Background: Humans excel at recognizing speech in natural auditory scenes despite large variation in speakers’ voice quality, intonation, and pitch. We previously demonstrated that different types of background sounds and their spectrotemporal statistics can improve or worsen speech recognition accuracy (Clonan et al 2025). However, how foreground speech features, such as fundamental frequency or formants, influence speech recognition accuracy is not well understood.

Methods: Here we explore how different speech articulatory features interact with natural and perturbed background sounds to influence speech recognition abilities. Participants (n=10) were required to recognize digit sequences (e.g., 0-9-1, 2-5-7) generated by adult and adolescent male

and female talkers and then mixed with various natural background sounds (e.g., water, babble, construction noise etc.; SNR= -9 dB) and perturbed background variants with distorted spectrum or modulation statistics. To characterize the unique interactions between the foreground and background sound statistical features, we used a hierarchical model of the auditory periphery and auditory midbrain, paired with generalized perceptual regression (GPR; Clonan et al 2025). GPR models perceptual judgments of single listeners with the neural-acoustic model and allows perceptual outcomes for arbitrary speech-foreground combinations to be predicted using auditory model features.

Results: We found that different spoken digits had different accuracies depending on both the background sound statistics and the speaker's voice. To explore these speech driven effects, we compared recognition abilities across the speech fundamental (F0), first formant (F1), and second formant (F2) frequencies, and harmonicity. Using a generalized linear mixed effects model, we found substantial, statistically significant trends for digit identity, F0, F1, and F2, on recognition accuracy, in addition to background-driven effects. Furthermore, we find that the neural inspired GPR model captures nearly 76% of the perceptual variance across background and digits. This model, based on spectral and temporal modulation statistics, successfully explains differences in recognition accuracy associated with individual talkers and talker identity (male, female, adult, adolescent) as well as differences associated with formant and harmonicity structure.

Conclusions: These results further identify foreground and background sound features that influence speech recognition in natural environmental noise and suggest that auditory computations involving modulations may underlie perceptual outcomes. Funded by NIH (R01DC020097).

SU193. Similarity and Divergence in Human and Machine Speech Recognition

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Background: Advances in machine learning have yielded machine systems that often approach human levels of performance on real-world perception tasks. For instance, models trained to recognize speech in noise reproduce some characteristics of human speech recognition. However, human and model speech behavior have not been extensively compared, leaving the extent to which such models can account for human speech perception unclear.

Methods: We measured word recognition abilities in humans and models across 37 types of speech distortions, spanning natural sources of distortion (e.g. background noise and reverberation) and artificial distortions (e.g. noise vocoding and audio filtering). We evaluated a set of industry speech recognition models along with in-house models. To investigate the factors that might give rise to human-like performance, we manipulated the architecture and the variability of training data for the in-house models. We measured the match between human and model performance across distortions.

Results: Two recent industry speech recognition models trained on hundreds of years of labeled speech (Whisper and Parakeet) exhibited high rank correlations with the pattern of human performance, but in absolute terms were superhuman on most types of distortion, and as a consequence were poor quantitative models of human speech recognition. In-house models with transformer-based architectures trained on a dataset that was intended to be more realistic in size (around 1400 hours) and variability produced better quantitative matches to humans. The match to human behavior was worse for models trained on less variable speech data, and for models that had exclusively convolutional architectures, even when they were matched in number of parameters.

Conclusions: The results are consistent with the idea that the robustness of human speech recognition results from the variability of real-world experience, but suggest that the computational architecture contributes to obtaining human-like generalization. Overall, the results show that superhuman performance is possible with systems that are optimized over enough data, and indicate that accurate models of human speech recognition can be obtained by optimizing models over the amounts and types of speech audio that humans are plausibly exposed to over developmental timescales.

SU194. Benchmarking Artificial Neural Network Models of Human Auditory Processing With Sound Similarity Judgements

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Background: With the increasing use of artificial neural networks (ANNs) in psychology and neuroscience, it is critical to pick appropriate benchmarks to assess how well such networks capture aspects of human perception and cognition. Most of this development has occurred in the visual domain, thus the generalizability of these visual model benchmarks to other sensory domains remains unclear. Here, we explored one possible benchmark: the extent to which human similarity judgments could be used to predict model-brain alignment across a broad range of ANNs trained on auditory tasks.

Methods: From a set of 20 ANNs, we computed cosine similarity scores between model activations for pairs of sounds. Sounds came from a dataset we compiled to be representative of sounds encountered in daily life. Models varied in architecture, training data, and training task, however none were trained on a similarity task. We calculated Spearman correlation between each models' similarity scores and sound pair similarity judgements from human participants. Additionally, we compared these correlations to a neural model-brain alignment metric from fMRI data to investigate whether models that best captured human similarity judgments also explained the most variance in neural data.

Results: The strength of correlation between similarity metrics computed from ANNs and human similarity judgements varied across models, though some ANNs did approach the human-to-human correlation ceiling. In general, ANNs trained on multiple tasks or trained on audio event categories had higher correlations to human similarity measures. Furthermore, the extent to which a given ANN captured human-like similarity judgements was strongly correlated with how well the ANN captured neural firing in its layer activations, but there was some unique variability in each measure.

Conclusions: The correlation between model similarity structure and human similarity judgments offers a task-agnostic benchmark for evaluating brain-model alignment. The variability across different models suggests that the metric has sensitivity to distinguish different models. Much like networks trained on vision tasks, some auditory ANNs captured human similarity judgements to a high degree, suggesting that these models share some auditory representational features with humans. Brain-model alignment measured with this similarity benchmark was positively correlated with a model's ability to capture neural firing patterns in its activations; therefore, this benchmark could potentially be used as an exploratory precursor for cortical brain-model alignment studies.

SU195. Hearing-In-Noise Problems Within a Large Online-Recruited Cohort

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Background: The primary clinical measure for hearing loss is the audiogram. However, suprathreshold hearing-in-noise problems only correlate moderately with absolute hearing thresholds. Consequently, audiometric thresholds fail to capture enough information for an individual's hearing performance characterization (Kollmeier, 1999, *Psychophysics, physiology and models of hearing*, pp. 211-218). There is even a considerable percentage of the population that has hearing-in-noise problems despite clinically normal audiometric thresholds, loosely categorized by terms such as "hidden hearing loss" or "central auditory processing disorder" (Schaette and McAlpine, 2011, *J Neurosci.*, 31(38), pp. 13452-7; Tremblay et al., 2015, *Ear and hearing*, 36(6), e290-e299). We ran a lab study measuring audiometric thresholds as well as hearing-in-noise to characterize an individual's hearing to find emerging measurements or model parameters for hearing characterization. In a further step, we introduced remote testing with a large participant cohort (N = 627, 4 different languages) measuring the same hearing-in-noise tests as in the lab experiments to enlarge sample size and to meaningfully identify types or principal components of suprathreshold hearing and to evaluate online testing procedures in a broader test battery.

Methods: Audiometric thresholds, loudness growth functions and tone- as well as speech-in-noise thresholds in 50 participants with age-typical or mild hearing loss who had no hearing aid experience were measured in the lab. 24 participants from the lab study also participated in remote testing to form a validation group to compare lab and remote test results. Moreover, 627 participants took part in remote testing.

Results: Correlations and RMS error-analysis revealed tone-in-notched-noise- and N0S π thresholds as potential candidates to characterize an individual's hearing by predicting numerous tone- and speech-in-noise measurements with high accuracy in the lab study. A single 500-Hz N0S π threshold explains 61% of the variance in diotic speech-in-noise performance (RMS error = 0.80 dB). Including remote test results revealed a highly significant increase for all tone-in-noise thresholds for the 24 participants taking part in both the lab and remote experiments. Largest differences were found in the aforementioned most diagnostic stimuli: tone-in-notched noise as well as dichotic tone-in-noise detection. On a population level, results were similar for personally recruited compared to online-recruited participants.

Conclusions: Tone-in-notched-noise and N0S π thresholds turned out to be promising candidates for a quick screening of hearing-in-noise abilities in clinical practice. Significantly worse remote tone-in-noise thresholds indicate the need for additional care when designing online hearing tests, especially for the most cue-specific stimuli. Nonetheless, similar remote testing results for known and unknown test participants indicate the usefulness of online recruitment for epidemiological studies of hearing problems. These results showcase the possibilities of remote hearing testing using tone-in-noise thresholds for a qualitative diagnostic of suprathreshold hearing in an efficient and cost-effective way.

SU196. Exploring the Link Between Frequency Selectivity and Temporal-Coherence–Based Speech Segregation in Sensorineural Hearing Loss

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Background: Individuals with hearing loss commonly report trouble hearing in noise despite using prescriptive amplification. Listening in noise depends on our brains' ability to parse an auditory scene and segregate different sound sources. A key mechanism thought to support segregation is grouping of across-frequency acoustic elements that are temporally coherent (in-phase or comodulated) to form unified perceptual streams or objects. Prior computational modeling work suggests that reduced frequency selectivity, as is common in sensorineural hearing loss (SNHL), can degrade temporal-coherence-based segregation. However, whether broader tuning in SNHL covaries with temporal-coherence-based masking release for speech from background noise has not been directly tested.

Methods: We collected the following measures from native speakers of North American English aged 18-65 years across the continuum of normal-hearing and symmetric SNHL: (i) psychophysical tuning curves (at 4 kHz), (ii) comodulation masking release (CMR) measured for 4 kHz tones in narrow bands of noise, and (iii) speech-in-noise scores while manipulating the temporal coherence between frequency bands containing speech and noise. To date, 5 normal-hearing participants and 6 SNHL participants have completed this study. Importantly, all psychophysical measures used stimuli that were amplified to restore normal loudness perception using a linear hearing aid (Moore and Glasberg, 1998).

Results: Preliminary data suggest that CMR for tonal targets is smaller in listeners with SNHL compared to those with normal hearing. Furthermore, individuals with SNHL also exhibit broader psychophysical tuning-curve tips and reduced tip-to-tail ratios. With data collection and analysis ongoing, individual tuning-curve metrics will be compared to individual temporal-coherence-based masking release data for both speech-based and tone-based stimuli.

Conclusions: We seek to collect data from N=30 participants to test the hypothesis that reduced frequency selectivity covaries with temporal-coherence-based masking release even when audibility is restored.

SU197. Neural Changes After Insc Transplantation Into the Scala Tympani in an Auditory Neuropathy Model

So-Young Chang^{*1}, Jae Yun Jung², Hosup Shim², Ji Eun Choi², Min Young Lee²

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Background: Studies investigating cochlear hair cell regeneration have been conducted worldwide. However, only a few have been successful, and the clinical application of these therapeutic approaches remains uncertain. Recent advances in cochlear implant techniques are impressive, but maintaining cochlear neural health to improve cochlear implant outcomes remains challenging due to secondary degeneration. Recent studies have demonstrated the direct conversion of mouse somatic cells into induced neural stem cells (iNSCs), which may provide an unlimited source of neuronal cells for autologous transplantation in patients with neurological disorders.

In this study, we evaluated the effects of iNSC transplantation on auditory neural connections in an animal model of auditory neuropathy induced by ototoxic drugs.

Methods: Mongolian gerbils aged 8–9 weeks and GFP-tagged human iNSCs kindly donated by Prof. Shim³ were used. For auditory nerve denervation, 1 mM ouabain solution soaked in gelfoam was placed on the round window (RW) for 30 minutes. Two weeks after ouabain application, human iNSC-GFPs at a density of 6×10^4 cells per 3 μ L were injected into the scala tympani (ST) using a cannula. Auditory function was assessed via auditory brainstem response (ABR) before and after ouabain treatment and surgery. Two weeks after hiNSC transplantation, ABR and electrical ABR (eABR) were recorded, and animals were sacrificed. Hematoxylin and eosin (H and E) staining, epifluorescence analysis of GFP, NF, TuJ, CtBP2, and VGLUT1, as well as quantification of spiral ganglion neurons (SGNs) in the cochlea, were performed.

Results: Ouabain application resulted in hair cells loss and increased ABR thresholds compared to controls. Two weeks after hiNSC transplantation, transplanted iNSCs were located in Rosenthal's canal and increased the neural cell population. ABR thresholds showed no improvement at this time point. The organ of Corti displayed less organized nerve fibers and synaptic structures, with wider intercellular gaps. The average distance between nerve fibers and presynaptic puncta was significantly greater in the hiNSC group. In the central auditory pathway, VGLUT1 expression was significantly increased in both cochlear nuclei, and eABR recordings revealed delayed action potentials in the hiNSC group, indicating partial recovery of afferent neural connections.

Conclusions: Human iNSC transplantation restored neural structures after degeneration, significantly enhancing synaptic connections toward the central auditory pathway, although peripheral synapses were not improved. These findings suggest that combining autologous stem cell transplantation with cochlear implants may improve outcomes and enable cochlear implant use in patients with long-term hearing loss.

SU198. Epigenetic Regulation of EDNRB2, a Key Regulator of Transdifferentiation of Supporting Cells Into Hair Cells, in Explants of Chick Auditory Epithelia

Mami Matsunaga^{*1}, Marie Takeuchi¹, Kei Ida¹, Koji Nishimura¹, Yosuke Tona¹, Koichi Omori¹, Takayuki Nakagawa¹

¹Kyoto University

Background: Our previous studies demonstrated that EDNRB2 regulates fate determination of reprogrammed supporting cells during transdifferentiation of supporting cells into hair cells in collaboration with ATOH1 in chick basilar papilla explants (Takeuchi et al., 2025). This study aimed to elucidate the upstream signals for EDNRB2 expression during hair cell regeneration in chick basilar papilla explants using ATAC sequencing.

Methods: We performed RNA- and -ATAC seq using our explant culture model, in which HCs are entirely lost by exposure to streptomycin (SM) for 48 h. Time course samples for RNA- and ATAC-seq are SM0 (before SM exposure), SM24 (24-h exposure to SM), and SM48 (48-h exposure to SM). For sampling, 25-35 basilar papillae were used for one batch and prepared in duplicate for both analyses. We performed transcription factor (TF)-binding motif scanning for detected enhancer-candidate loci. Based on this, in situ hybridization or immunostaining was done to examine the expression patterns of candidate TFs.

Results: RNA-seq identified 24,888 genes, and ATAC-seq identified 80,366 open chromatin lesions. We identified three candidate loci for EDNRB2 enhancer lesions. TF-binding motif scanning revealed 98 candidate motifs. Filtering by the expression levels of candidate TFs in RNA sequencing data, 23 candidate TFs were identified. Previous studies (Matsunaga et al., 2020; Janesick et al., 2022) have demonstrated upregulation of JAK-STAT signaling pathways in the initial process of hair cell regeneration in chick basilar papillae. Based on this, we chose a couple of TFs, which are associated with JAK-STAT signaling, from 23 candidate TFs for histological evaluation. Phosphorylated STA3 exhibited a similar distribution to EDNRB2 expression.

Conclusions: We conducted an integrated analysis of RNA and ATAC sequencing to explore candidate upstream TFs for EDNRB2. Based on histological findings, functional analyses of candidate TFs will be done in the near future.

SU199. Reducing Sox2 Increases Cochlear Supporting Cell Reprogramming Efficiency

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Background: Sox2 is dynamically expressed in the developing inner ear and expressed in mature supporting cells but not in hair cells. Germline Sox2 haploinsufficiency induces supernumerary hair cells and enhances natural hair cell regeneration in the neonatal cochlea. Mature supporting cells can be reprogrammed to hair cell like cells by combined expression of the transcription factors Atoh1, Gfi1, and Pou4f3 (GAP). To test whether Sox2 influences reprogramming efficiency, we activated GAP and reduced Sox2 in mature supporting cells.

Methods: We bred GAP overexpressing mice to mice carrying either a Sox2 allele flanked by LoxP sites or wild-type Sox2 and crossed these with Pou4f3-DTR mice, to kill hair cells, and Ai3 reporter mice, to track cell lineage. Cre was introduced by intra-labyrinthine AAV-Lfng-CreER or by crossing with Lfng-CreER transgenic mice. Cre recombination with tamoxifen at 3 or 6 weeks induced GAP reprogramming, as well as knocking out of a Sox2 allele. Hair cells

were killed with diphtheria toxin. Mice were euthanized 2 or 9 weeks after hair cell death, and their inner ears harvested for quantitative immunofluorescence microscopy.

Results: Simultaneously reducing Sox2 and overexpressing GAP significantly increased the number of reprogrammed hair cells by 50% at 2 weeks, and by 100% at 9 weeks compared to GAP controls. Reprogrammed hair cells appeared immature and quantifying stereocilia with phalloidin staining showed no difference between genotypes.

Conclusions: Reducing Sox2 in supporting cells at the time of GAP activation significantly increased the number of reprogrammed hair cells. Future therapies for sensorineural hearing loss relying on reprogramming supporting cells could incorporate Sox2 decreasing strategies to increase reprogramming efficiency.

SU200. Exploring Spontaneous Regeneration of Spiral Ganglion Neurons Post-Ablation

Joshua Lin^{*1}, Nhi Nguyen¹, Priya Shah¹, Seiji B. Shibata¹

¹*Keck School of Medicine of USC*

Background: The current consensus suggests that spiral ganglion neurons (SGN) do not spontaneously regenerate in mammals. A recent study has reported spontaneous SGN regeneration following ouabain treatment in adult mice, demonstrating the upregulation of glia markers Sox2/Sox10 within cells expressing the neuronal marker Tuj1. Yet, the precise origin of the spontaneously regenerated SGNs remains unclear. As such, we utilized an inducible Sox2Cre-ER mouse model to enable lineage tracing following SGN ablation with diphtheria toxin (DT).

Methods: Wild-type (C57) and Sox2Cre-ER crossed with Ai9-TdTomato Cre-reporter mice (Sox2Cre-ER+/Td+) obtained from the Jackson Laboratory were used. A single injection of AAV-retro encoding diphtheria toxin receptor gene with GFP (AAV-DTR) was injected via the posterior semicircular canal (PSCC) at P1-2 to express DTR in the SGN. We observed near-total SGN transfection without transfecting the hair cells as early as 7 days post-injection. At 7 or 28 days post-AAV-DTR inoculation, neonatal (P9) and adult (P30) mice were administered DT at 5ng/g and 20ng/g, respectively. Distortion-product otoacoustic emissions (DPOAEs) and auditory brainstem responses (ABRs) were measured before histological assessment and immunohistochemistry of harvested cochleae at 14 days after DT administration. We performed lineage tracing experiments in Sox2Cre-ER+/TdTomato+ mice injected with AAV-DTR at P1-2. At 7 or 28 days post-AAV-DTR inoculation, Tamoxifen was introduced to activate Cre recombinase expression with DT. AAV-DTR-injected, age-matched, saline-administered mice served as controls.

Results: The AAV-DTR-injected wild-type mice, receiving 5ng/g DT at P9 (neonate) and 20ng/g DT at P28 (adult), showed normal DPOAE and increased ABR thresholds at 14 days, consistent with auditory neuropathy. Saline-administered controls demonstrated normal DPOAE and ABR thresholds. Subsequent histology demonstrated a 94% reduction in Tuj+ SGN density (203.86 cells/mm²) in neonates and a 78% reduction in Tuj+ SGN density (894.43 cells/mm²) in adults. Immunohistochemistry indicated co-localization of Tuj and Sox2 signals in 6% of the surviving SGNs in both wild-type deafened neonatal and adult mice, consistent with previous literature. Our preliminary lineage tracing experiment in AAV-DTR-injected Sox2CreER+/Td+

mice demonstrated similar levels of SGN ablation by 14 days after DT administration (92% in neonates and 91% in adults). Consistent with the wild-type immunohistology, we observed TdTomato signals in the few surviving Tuj+ cells in both neonatal and adult mice, indicating spontaneous transdifferentiation from the glial cell lineage to exhibit a neuronal phenotype.

Conclusions: We established a new auditory neuropathy model with temporal control: introduction of 5 and 20ng/g of DT in AAV-DTR-injected mice was sufficient to ablate 78-94% of SGN in neonatal (P9) and adult (P30) mice, sparing the hair cells. Our preliminary fate-mapping experiments in AAV-DTR-injected Sox2Cre-ER+/TdTomato+ mice indicate that spontaneous SGN regeneration from Sox2-positive glial cells occurs in both the neonatal and adult mice following DT-induced SGN ablation. We will further quantify and characterize the regenerated SGN in future experiments.

SU201. Exploring Genes that Enable Hair Cell Regeneration in Zebrafish

Andrea Meza Hernandez*¹

¹*Johns Hopkins University*

Background: Hair cells are mechanosensory receptors of the inner ear that are essential for hearing and balance. Damage or loss of these cells in mammals results in permanent hearing loss and vestibular dysfunction due to limited regenerative capacity. In contrast, zebrafish and other non-mammalian vertebrates are able to robustly regenerate hair cells following injury. Understanding the molecular mechanisms that enable hair cell regeneration in these systems may reveal

conserved genetic pathways that could be therapeutically targeted to restore hearing in mammals.

Recent in vitro studies using mammalian inner ear organoid cultures have identified a set of genes implicated in hair cell regeneration through bulk RNA sequencing and single-cell RNA sequencing approaches (Li et al., 2022). These analyses revealed six key regulatory factors: Trim71, Arid3a, Jarid2, Rprm, Nfix, and Zbtb20. These factors are enriched under regenerating conditions compared to controls. They are proposed to play roles in reprogramming supporting cells and promoting hair cell regeneration. While these findings provide important insight, the functional roles of these candidate genes in vivo remain largely unexplored.

This project aims to investigate the in vivo function of zebrafish homologs of selected candidate regeneration genes using the zebrafish larval lateral line system. In addition to possessing hair cells in the inner ear, zebrafish have external accessible hair cells located along their body surface in the lateral line, making them a powerful model for studying hair cell damage and regeneration. Hair cells in the lateral line can be selectively ablated by exposing larval zebrafish to ototoxic compounds such as copper sulfate without damaging surrounding tissues.

Methods: To assess the role of candidate genes in hair cell regeneration, CRISPR/Cas9 mediated mutagenesis will be used to generate zebrafish larvae with targeted gene disruptions. Five-day old CRISPR injected larvae will undergo a standard lateral line regeneration assay in which hair cells are ablated by a one-hour exposure to copper sulfate. Following ablation, larvae will be washed and allowed to recover for two days, a time point at which robust regeneration normally occurs. Regenerated hair cells will then be labeled using the vital dye YO-PRO-1 and quantified to identify defects or enhancements in regeneration relative to wild type controls.

Results: Hair cells count from copper treated CRISPR injected mutants will be compared to untreated CRISPR injected larvae, copper treated wildtype larvae, and untreated wildtype controls. Following phenotypic analysis, genomic DNA will be isolated from individual larvae and analyzed by PCR and sequencing to confirm CRISPR-induced insertions or deletions at the targeted loci.

Conclusions: Together, this work seeks to define the functional roles of conserved regeneration_x0002_- associated genes in vivo and to identify genetic regulators that enable hair cell regeneration.

These findings may advance our understanding of sensory regeneration and inform future strategies for restoring hearing in mammals.

SU202. Processing of Salient Vocalizations in Auditory Cortical Regions Cell Types

Zahra Ghasemahmad^{*2}, Elliot Chin¹, Maryse Thomas³, Carolyn Sweeney³, Jeffrey Wenstrup³, Anne Takesian²

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Background: Mice produce a rich repertoire of complex vocalizations across diverse behavioral contexts, such as mating and stress. Playback of these calls elicits distinct behavioral responses and neuromodulator release patterns that vary with the listener's state and the context in which the calls occur. The auditory cortex is thought to relay information about these vocalizations to motor and limbic centers, thereby shaping context-appropriate reactions. However, how such salient vocalizations are represented across cortical subpopulations, and how this representation depends on behavioral state, remain unclear.

Methods: We measured vocalization responses in multiple auditory cortical cell types, including pyramidal excitatory neurons and a subset of inhibitory interneurons expressing Neuron-Derived Neurotrophic Factor (NDNF). These interneurons receive strong neuromodulatory inputs and are thought to modulate cortical activity in a state-dependent manner. Using transgenic mice expressing GCaMP in either pyramidal neurons or NDNF interneurons, we performed widefield and two-photon calcium imaging across auditory cortical regions in awake, head-fixed mice. Mice listened to playback of vocalization syllables recorded during negative (stress), positive

(mating), or neutral (isolation) contexts, while we simultaneously monitored pupil dynamics and locomotion.

Results: Vocalizations evoked robust, widespread responses across primary auditory cortex (A1) and multiple higher-order regions. Two-photon recordings revealed region- and cell type-specific response patterns, with subsets of L2/3 and L4 pyramidal neurons, and NDNF interneurons displaying strong selectivity for specific vocalizations or call classes (e.g., mating versus stress). Notably, many neurons responded robustly to natural vocalizations but not to synthetic sounds with matched spectral content, and small ensembles of vocalization-selective neurons could reliably decode call identity. Ongoing analyses are evaluating how behavioral state modulates the selectivity of these responses.

Conclusions: Together, these findings suggest that auditory cortical circuits encode behaviorally relevant vocalizations in a region- and cell-type-specific manner, and highlight a role for NDNF interneurons in shaping state-dependent processing. By identifying circuit mechanisms for encoding natural communication sounds, this work advances our understanding of auditory perception and lays a foundation for future studies of how internal state and experience shape communication.

SU203. Deep-Neural-Network-Based Hearing Loss Compensation Methods: Personalization and Patient Benefit

Sarah Verhulst¹, Chuan Wen¹, Marjoleen Wouters¹, Morgan Thienpont¹, Nele De Poortere¹, Attila Fráter¹, Guy Torfs¹

¹*Ghent University*

Background: Deep neural network (DNN)-based approaches are increasingly being used to design hearing-loss compensation algorithms. These methods offer low-latency, end-to-end processing suitable for deployment on resource-constrained devices, and they provide greater flexibility in addressing various aspects of sensorineural hearing loss (SNHL) compared to traditional techniques. Their nonlinear structure and ability to optimize via backpropagation make them particularly effective in compensating for deficits caused by cochlear synaptopathy (CS) and outer hair cell (OHC) damage.

Methods: We propose a closed-loop framework for developing augmented-hearing algorithms by minimizing the loss between outputs of differentiable biophysical models simulating normal and impaired auditory-nerve responses. The resulting CoNNear-HA algorithms are personalized using the individual's audiogram and CS profile inferred from auditory physiology. We first benchmark these personalized algorithms against conventional methods using standardized tests, including transfer functions and step/impulse responses. We then evaluate their impact on speech intelligibility in noise while ensuring sound quality remains acceptable. **Results:** We report speech recognition thresholds (SRTs) to 70-dB Flemish Matrix sentences in stationary noise for 20 subjects treated with either CS-specific or combined CS-OHC CoNNear-HA algorithms. While not all participants showed improvement, individual outcomes were analyzed in relation to their audiogram and envelope-following response strength. Post-hoc analysis revealed that the greatest benefit occurred in individuals whose baseline SRTs deviated most from the normal-hearing reference group. To assess performance in a more challenging listening scenario, we tested 40 subjects (20 older normal-hearing and 20 hearing-impaired) on a word recognition task

in babble noise using a signal-to-noise ratio corresponding to their unprocessed SRT. In this condition, most participants benefited from the CoNNear based processing.

Conclusions:

These findings support the integration of DNN-based algorithms alongside conventional methods to enhance hearing in individuals with early to advanced SNHL.

Work supported by FWO G063821N (Machine Hearing 2.0) and EIC transition grant 101058278 (EarDiTech)

Late Breaking Podium

3:00 p.m. - 4:00 p.m.

Meeting Room 209ABC, Floor 2

Moderators: Jeffrey R. Holt and Ronna Hertzano

Molecular Drivers of Directional Regeneration of Spiral Ganglion Neurites and Their Therapeutic Application in Synaptopathy

3:00 p.m. - 3:15 p.m.

Volkan Ergin¹, Wei Wei¹, Chenxi Jin¹, Yi-Zhou Quan¹, Wan Du¹, Stewart Silver¹, Natalie Pelon¹, ZhengYi Chen^{*1}

¹*Massachusetts Eye and Ear, Harvard Medical School*

Background: Spiral ganglion nerve (SGN) degeneration underlies word recognition deficiency and poor cochlear implant (CI) outcomes. While neurotrophic factors, such as BDNF and NT3, promote general neurite growth, their roles in the directional regeneration of spiral neurites and their effect on synaptopathy have been limited. In adult cochlea, while the detachment and retraction of spiral neurites from inner hair cells is generally permanent, we found that adult SGN can regrow their neurites in response to regenerated hair cells (HCs) to form connections. We hypothesized that regenerated HCs secrete molecules to promote this directional regeneration of adult neurites and their identification holds great promise for treating synaptopathy and improving CI performance.

Methods: We used single-cell RNAseq (ScRNAseq) to study transcriptomes in regenerated HCs via reprogramming and ATOH1 overexpression. Bioinformatics was used to identify molecules secreted by regenerated HCs. SGN explant culture assays were used to assess neurite regeneration induced by individual and combined candidates. Dose response and antibody blockade were used to study specificity and potency. In vivo effect on neurite regeneration was studied using noise-exposed and ouabain-treated mouse models with permanent spiral nerve degeneration. AAV-mediated gene therapy was used to deliver the candidates into mice exposed to noise or during aging. ABR thresholds and wave 1 amplitudes were measured with synapses labeled and quantified.

Results: ScRNAseq identified 70 molecules secreted by regenerated HCs, which were reduced to 14 high-probability candidates by bioinformatics analysis. Testing showed a three-molecule combination of Col18a1, Sparc, and Igfbp2 (CSI) to be potent on neurite growth that is dose-dependent and can be specifically blocked by C, S and I antibodies. In adult cochlear explant culture, CSI successfully promoted neurite regeneration and connections with regenerated HCs. Biochemically, C, S and I were shown to interact intracellularly but not as secreted forms. AAV-CSI delivery in adult cochlea, after noise exposure or ouabain treatment, led to potent, directional neurite regeneration toward HCs to form connections. Significantly, AAV-CSI injection into adult inner ear, after noise-induced synaptopathy or with age-related synaptopathy, rescued wave 1 amplitudes and synapses. This rescue effect was mediated by neurite growth and synapse preservation.

Conclusions: Our work, stemming from adult HC regeneration, identified a new class of extracellular molecules COL18A, SPARC and IGFBP2 with potent roles in directional regeneration of damaged neurites in vitro and in vivo. CSI gene therapy achieved therapeutic outcomes in mouse models of NIHL and ARHL. We identified a target-initiated mechanism for neurite regeneration distinct from developmental mechanisms and revealed the regeneration capacity of damaged adult neurites. CSI represents a promising strategy to treat auditory synaptopathy, improve CI outcomes and enhance the treatment of transplanted neuro-stem cells for auditory neuropathy.

Selective Alterations in Intrinsic Excitability of Neurons in the Superior Olivary Complex in a Rat Model of Autism

3:15 p.m. - 3:30 p.m.

Samantha Lauby¹, Aaron Hardman¹, Shaam Anand¹, Nace Golding¹

¹*University of Texas at Austin*

Background: Auditory processing difficulties are often reported from individuals with neurodevelopmental disorders, including autism spectrum disorder. Changes in auditory brainstem functioning, including auditory brainstem responses (ABRs), precede other symptoms of autism spectrum disorder and thus may be a useful biomarker for early detection and intervention. However, the underlying biological mechanisms underlying these changes in ABRs in human infants is largely unknown. Prior research in the autistic brain, especially the cortex, has consistently reported alterations in excitatory/inhibitory balance as a potential biological mechanism underlying the disorder and alterations in auditory brainstem nuclei have also been reported.

Methods: We used a prenatal valproic acid (VPA) exposure model of autism in rats to examine changes in ABRs at postnatal days 35-37. Waveform amplitudes were measured as well as the dB threshold for broadband click and tone stimuli (4-48kHz). Ex vivo patch clamp recordings were made in whole neurons in the superior olivary complex of the auditory brainstem from postnatal days 29-35. Intrinsic membrane properties, including rheobase, input resistance, and time constant were measured in current clamp mode. HCN channel functioning via sag ratio was also assessed in current clamp and their conductance and biophysical properties were assessed in voltage clamp mode. The morphology of the patched neurons was also assessed.

Results: We found that prenatal VPA-exposed offspring have an elevated ABR threshold at all tone stimuli (but not clicks). We also found stimulus-dependent changes in Wave III amplitudes and increased Wave IV/I ratio at all stimuli. At the single-cell level, we found an elevated rheobase, shorter time constant, and higher sag ratio in principal neurons in the medial nucleus of the trapezoid body. This was associated with a higher maximum conductance of HCN channels, suggesting an increase in the total number of HCN channels. We did not find any major alterations in intrinsic membrane properties in principal neurons in the lateral superior olive.

Conclusions: Our results suggest that prenatal VPA-exposed offspring have alterations in central gain and excitatory/inhibitory balance at the superior olivary complex, which can be identified through ABRs. VPA can alter the expression of many genes, so the increase in the maximum conductance of HCN channels in the medial nucleus of the trapezoid body might reflect an underlying mechanism that links prenatal VPA exposure to impaired inhibition in this brain region. Deficits in the detection of speech patterns in challenging listening environments are well-known hallmarks of autism spectrum disorders in humans. The changes we have identified in the sound localization circuitry of the lateral superior olive of rats may provide mechanistic insight into these deficits, and help guide potential strategies for remediation.

A Vestibulothalamic Relay to Posterior Parietal Cortex Defines a Canonical Vestibular Neuraxis

3:30 p.m. - 3:45 p.m.

Todd Mowery*¹, Mark Aziz¹, Justin Yao¹

¹*Rutgers University*

Background: The striatum is the primary input nucleus of the basal ganglia, integrating dense corticostriatal and thalamostriatal projections to regulate action selection and learning. Extensive comparative neuroanatomical work across rodents, nonhuman primates, and carnivores has revealed robust sensorimotor substrates for motor, somatosensory, auditory, and visual processing. In contrast, vestibular sensory pathways remain comparatively unexplored. Our recent work identified a major vestibulothalamic relay from the medial vestibular nucleus (MVN) to the parafascicular nucleus, revealing extensive sensorimotor overlap and a compartmentalized projection zone in the dorsoventral striatum.

Methods: Using retrograde and anterograde AAV tracing in adult Mongolian gerbils, we systematically mapped a second ascending vestibular pathway. Retrograde AAV-GFP (350 nL) was pressure-injected (50 nl/minute) using a Nanoject III at stereotaxic coordinates targeting the center of the lateral dorsal (LD) thalamic nucleus and posterior parietal cortex (PPC). The pipette was left in place for 20 minutes following injection. Anterograde AAV1-GFP (350 nL) was similarly delivered to the center of LD. After three weeks of viral expression, animals were perfused, and brains were extracted, post-fixed, and sectioned coronally at 50 μ m. Sections were float-mounted onto pig-gelatin-coated slides and coverslipped with DAPI-containing mounting medium. All sections were imaged on an Echo Revolve R4 fluorescence microscope using matched exposure and gain settings. Mean fluorescence intensity (arbitrary units) was quantified using the ImageJ histogram tool and exported to Excel. Fluorescence levels were measured

across brainstem, thalamus, cortical, and striatal regions and analyzed in JMP using one-way ANOVA with Tukey's HSD post-hoc comparisons.

Results: We find that projections from MVN target the lateral dorsal (LD) nucleus of the thalamus, which provides a robust thalamocortical input to the posterior parietal cortex (PPC). In turn, PPC corticostriatal projections terminate in a compartmentalized region of the dorsolateral striatum, mirroring the organizational features of the previously described MVN→PF→striatum pathway. A PPC specific compartment just dorsal to auditory and visual inputs to the dorsolateral striatum could represent vestibular striatum.

Conclusions: Together, these results identify a previously unrecognized vestibular circuit linking brainstem, thalamus, parietal cortex, and striatum—defining a canonical vestibular neuraxis in rodents. This pathway provides a new framework for studying vestibular contributions to basal ganglia physiology, cross-modal plasticity, and behavior across species.

Nonclinical Development of a Therapeutic for Usher Syndrome Type 3A Associated Hearing Loss

3:45 p.m. - 4:00 p.m.

Katherine Gribble*¹, Yuan Gao¹, Ye-Hyun Kim¹, Matthew McGill¹, Yukako Asai¹, Lars Becker¹, Vikrant Borse¹, Junaid Syed¹, Janmaris Marin Fermin¹, Izra Hernandez Rivera¹, Michael McKenna¹, Brian Lin¹, Neda Masoudi¹, Bifeng Pan¹, Jean Phillips¹, Kathleen Lennon¹, Danielle Lenz¹, Eva Andres-Mateos¹, Michelle Valero¹

¹*Eli Lilly and Company*

Background: Usher Syndrome Type 3A (USH3A) is an inherited genetic condition affecting hearing and vision. Individuals with USH3A experience progressive bilateral loss of hearing and vision, with clinical diagnosis typically occurring by the age of 10 years. Hearing loss in USH3A is typically symmetric, sensorineural, and progressive, often reaching the level of bilateral profound sensorineural hearing loss (SNHL) by the third or fourth decade of life.

Although USH3A is believed to be caused by a defective clarin-1 protein (individuals with variants in the clarin-1 gene present with USH3A), there are currently no approved pharmacologic therapies for treating either the progressive hearing or vision loss associated with USH3A. The only treatment options for USH3A-associated hearing loss are hearing aids and cochlear implantation, neither of which provide the opportunity to recover lost physiologic hearing nor to halt further progression of physiologic hearing loss. We have developed a therapy that can be delivered to the cochlea to address USH3A hearing loss as discussed below.

Methods: Here we present nonclinical data from mice and non-human primates (NHPs) supporting development of an USH3A therapy for hearing loss. Our USH3A therapy is designed for local delivery to the cochlea. We first characterized the auditory phenotype and disease progression of a mouse model to evaluate its relevance to the USH3A-mediated hearing loss phenotype in humans. Auditory and cochlear function were assessed with auditory brainstem response and distortion-product otoacoustic emission testing, respectively. Cochlear histologic

evaluations were performed to evaluate hair cell survival and sub-cellular hair bundle dysmorphology. We then evaluated the potential for efficacy and otic tolerability of our USH3A therapy across multiple doses when administered at an age when the murine cochlea is mature but before substantial degeneration of hair cells occurs. In NHPs with mature cochleae, we evaluated otic tolerability of our USH3A therapy across multiple doses.

Results: In a mouse model of USH3A-associated hearing loss, we observed a dose-dependent improvement in auditory and cochlear function following administration of our USH3A therapy. Additionally, we observed that increasing doses of our USH3A therapy are well tolerated in the mouse and non-human primate cochlea.

Conclusions: Our results in mice and NHPs support the development of our USH3A therapy as a potential treatment for individuals with USH3A-associated hearing loss.

Symposium 2: New Neurostimulation Strategies for Auditory Restoration

3:00 p.m. - 5:00 p.m.

Ballroom B, Floor 3

New Neurostimulation Strategies for Auditory Restoration

Brice Bathellier, *Institut Pasteur, Institut Audition*

Symposium Description: The cochlear implant (CI) is by far the most successful sensory restoration device, putting the hearing research at the forefront of neurostimulation device development. CI have shortcomings, including their restriction to patients with preserved auditory nerve, and their poor spectral accuracy. Intense research is currently on-going to solve these issues encompassing new algorithms or stimulation methodologies for CI, as well as the conception of brain targeted implants addressing the cochlear nucleus or the even the cortex. The aim of this symposium is to highlight recent progresses made on these fronts, including pre-clinical and clinical research. To account for the diversity of options considered in present research effort we will cover approaches developed at the level of the cochlea, brainstem and auditory cortex. Moreover, we will discuss a large range of research focuses including the impact of AI-based algorithms improving information transfer for CI users, or shaping information at higher level of the auditory system, the development of new electrical stimulators, microfabricated and flexible for higher resolution of stimulation and current progresses in the implementation of optogenetic stimulations techniques for auditory rehabilitation. The symposium will include established specialists and younger researchers demonstrating striking results that prefigure important technological steps with significant improvement of auditory rehabilitation, and will be organized into 6 talks of 15 min and one 30min talk (Moser).

Tania Barkat, *University of Basel*

New and Old Neurostimulation Strategies for Auditory Restoration

Julie Arenberg, *Massachusetts Eye and Ear Infirmary, Eaton Peabody Laboratory*

Individual Abstract: Our research addresses two main challenges that likely limit the speech perception outcomes for individuals with cochlear implants. The first challenge is providing temporal fine structure information. Current implants are primarily encoding the envelope of the acoustic signal, thereby discarding the fine temporal information. There is one strategy that attempts to encode this, but the evidence is mixed about how effective temporal fine structure is transmitted. The second challenge is providing spectral detail by stimulating more localized populations of auditory neurons. Current implants employ the monopolar electrode configuration that induces a broad spread of excitation and more unwanted channel interaction than can be achieved with focused electrical stimulation and potentially with magnetic stimulation through micro-coil arrays. This talk will demonstrate data from animal models demonstrating two approaches to solving these challenges. To provide temporal fine structure, we have applied continuous analog stimulation, which is how cochlear implants were stimulated when they were first developed, but we do this in combination with highly focused electrical fields. Results show that neurons in the inferior colliculus phase lock to the stimulus frequency, and two channels of simultaneous stimulation show small degrees of channel interaction as assessed by quantifying the degree of overlapping neural activation. To provide more spectral detail, we also explore magnetic stimulation from micro-coils of the auditory nerve. Intracochlear magnetic stimulation via a micro-coil produced robust IC activation; the spread of activation was significantly narrower vs. that from monopolar/bipolar electrical stimulation from implanted electrodes. These new stimulation approaches will be discussed within the context of standard cochlear implants in use today.

Bionic Hearing in Mammalian Species Through a Soft Auditory Cortex Implant

Alix Trouillet, *Centre hospitalier universitaire vaudois*

Individual Abstract: By 2050, nearly 700 million people will require hearing rehabilitation according to the World Health Organization. Cochlear implants (CI) are the first line of treatment for severe deafness and they restore meaningful sound perception and speech comprehension to both children and adults. However, significant limitations remain and underscore the urgent need for innovation: 1) CIs do not convey complex hearing experiences such as music or speech in noisy environments, 2) Outcomes are variable across patients with some poor responders with limited improvements, 3) CIs are not indicated for individuals with cochlear and auditory nerve abnormalities as well as central disorders. Direct electrical stimulation of the auditory cortex through an Auditory Cortex Implant (ACI) provides an alternative therapeutic approach tackling some of these challenges. We developed animal models enabling systematic assessment of ACI performance from electrophysiology and behavioral readouts. Applying this framework to rodents, minipigs and non-human primates (NHP), we demonstrated how ACI activates the auditory pathway in a tonotopic fashion, resembling acoustic stimulation. In NHP, the ACI electrode pitch determined the resolution of acoustic percepts enabling a rich encoding capacity of the device. Finally, we measured the behavioral responses to ACI and quantified the auditory nature of the percepts with its acuity and dynamic range. These results reveal the potential of auditory cortical stimulation to restore high-resolution hearing in patients with severe deafness.

Leveraging AI to Improve Speech Information Transmission With Cochlear Implants

Tobias Goehring, *University of Zurich*

Individual Abstract: Cochlear implants (CIs) help many people with severe-to-profound hearing loss hear better, but perceiving speech in a noisy or reverberant environment remains a major challenge for them. I will discuss the limitations in CI hearing responsible for these perceptual difficulties and then describe several avenues to leverage artificial intelligence (AI) to improve speech transmission and perceptual outcomes with CIs. AI methods for the CI frontend processor have shown unmatched potential for enhancing the intelligibility of speech in noisy situations. Our results from several studies using deep learning to improve speech-in-noise perception, include AI systems that can generalise to realistic acoustic situations, benefit from spatial information and let the user choose a specific speaker of interest at runtime. These deep-learning systems achieved meaningful and statistically significant improvements in speech intelligibility even in difficult situations with unseen noise and reverberation or with up to 3 concurrent speakers in background noise. I will present further work on AI-based approaches to tailor these systems specifically for CI speech processing and perception. A hybrid combination between computational models of the auditory system under electrical stimulation and their application as predictors and loss functions for the training of AI-based speech enhancement models is presented. Training such domain-aware speech enhancement models has the potential to further improve performance. Finally, the recent success of powerful speech foundation models in tasks such as automatic speech recognition and translation opens new venues to utilize large-scale speech models to directly assess and compare CI speech processing strategies. First results indicate the potential and feasibility of this approach by applying large speech models to probe speech information transmission in the context of CI simulation and speech processing strategies.

Toward Optogenetic Hearing Restoration

Tobias Moser, *University Medical Center Goettingen*

Individual Abstract: When hearing fails, cochlear implants (CIs) provide open speech perception to most of the currently more than a million CI users. CIs bypass the defective sensory organ and stimulate the auditory nerve electrically. The major bottleneck of current CIs is the poor coding of spectral information, which results from wide current spread from each electrode contact. As light can be more conveniently confined, optical stimulation of the auditory nerve presents a promising perspective for a fundamental advance of CIs. Developing optogenetic stimulation for auditory research and future hearing restoration requires concerted efforts toward development and characterization of appropriate channelrhodopsins, their viral gene transfer to cochlear neurons, as well as medical device engineering. The presentation will summarize the current state of the art and plans for developing gene therapy of the auditory nerve and optical CI as well as their combination for improved hearing restoration by optogenetic sound coding.

Cortical Implants for Hearing Restoration

Tania Barkat, *University of Basel*

Individual Abstract: Hearing loss affects half a billion people worldwide, significantly impairing communication and quality of life. While hearing aids provide adequate support for mild cases, severe hearing loss often requires cochlear implants. Despite their success, cochlear implants suffer from poor spectral accuracy, low acoustic resolution, and ineffectiveness in patients with auditory nerve damage. This study investigates whether cortical implants offer a more effective alternative for auditory restoration.

We implanted surface electrode arrays with 32 contacts (175 μm spacing) onto the auditory cortex of mice and compared them with cochlear implants featuring 4 electrodes (500 μm spacing). To ensure a direct comparison, we analyzed responses from four cortical electrodes, four cochlear implant electrodes, and four frequency-matched acoustic stimuli in normal-hearing mice. Mice were tested on single- and multi-frequency categorization tasks, amplitude modulation categorization, and amplitude modulation in noise categorization. Across all tasks, cortical implants led to superior auditory performance compared to cochlear implants, although learning took longer and followed distinct learning curves. Notably, mice trained with natural sounds could perform the tasks with cortical stimulation without additional training and vice versa, suggesting that cortical stimulation evokes percepts that closely resemble natural hearing.

To investigate the neural basis of these differences, we recorded in vivo electrophysiological responses in the auditory cortex to cortical stimulation, cochlear stimulation, and natural sounds. Neuronal responses scaled with stimulus strength across all conditions. Dimensionality reduction analyses (PCA, t-SNE) revealed that cortical stimulation responses resembled natural sound-evoked responses more closely than cochlear stimulation responses. Linear decoder analyses trained on natural sounds and tested on cortical and cochlear stimulation showed higher classification accuracy for cortical stimulation. Additionally, three-way decoder analyses confirmed that cortical stimulation responses were more similar to natural sounds than to cochlear stimulation, aligning with behavioral findings.

These results suggest that cortical stimulation not only provides better auditory performance but also more faithfully mimics natural auditory processing. The ability of mice to generalize between natural sound and cortical stimulation further supports this claim. While cortical implants show promise as an alternative for hearing restoration, future studies should further investigate learning dynamics, perceptual fidelity, and translational feasibility for clinical application.

A Generic Deep Learning Architecture for Bio-Inspired Encoding of an Auditory Cortical Implant

Brice Bathellier, *Institut Pasteur, Institut Audition*

Individual Abstract: Cochlear implants (CIs) are the most successful sensory neuroprosthesis and help almost a million patients to recover functional hearing. However, their relatively poor spectral and temporal accuracy limits speech intelligibility in noise and music perception. Moreover, CIs do not address pathologies involving auditory nerve loss or cochlear malformation. Because auditory cortex stimulation leads to sound perception, a possible approach to circumvent these issues could be to implant the neuroprosthesis at this level. However, cortical implants would require high-level encoding models to transfer information in a format similar to sound representations in the auditory cortex. To address this challenge, we have developed an AI-based encoder allowing us to recreate and map key features of the

biological auditory code observed in the cortex while ensuring a high information throughput, which is closely measured by reconstructing the waveforms of the encoded sounds. Our approach uses an autoencoder whose latent space matches the number of electrodes on the stimulator and the temporal resolution of auditory cortex activity. The autoencoder is combined with a classifier of human-labelled sound categories that enables the generation of structured pre-semantic representations. The encoder and decoder are based on modern 1D convolution layers explicitly developed for sound processing, yielding efficient training and excellent performance. We demonstrate that this novel hybrid architecture trained on millions of natural sounds can perform sound encoding on hundreds of channels with almost no information loss while generating stimulation patterns matching multiple neural code properties of the auditory cortex. Preservation of information is established by the high perceptual similarity of reconstructed sounds compared to original sounds, for speech, music and natural sounds. The similarity with natural auditory cortex representations is established by using representational similarity analysis on cortical data and on the representation of our encoder. In addition, specific constraints in the training process allow us to reproduce tonotopy. In addition, we demonstrate that single channel activities for well-known sounds have temporal profiles similar to cortical neurons with a variety of onset, offset and sustained responses, as well as tuning properties observed commonly in vivo. Finally, we show that mice can perceive and use the representations generated by our models with good precision. Hence, our approach allows for precisely delineating the design of a potentially successful auditory cortical implant for auditory restoration.

Distinct Cortical Neuronal Populations Are Tuned to Electrical and Acoustic Stimuli in a Chronic Soft Abi Mouse Model

Daniel Lee, *Massachusetts Eye and Ear Infirmary and Harvard Medical School*

Individual Abstract: The auditory brainstem implant (ABI) was developed to restore sound perception in individuals who are not candidates for cochlear implants (CI) due to retrocochlear hearing loss, such as those with Neurofibromatosis type 2 (NF2). Despite its initial promise, ABI performance remains limited—most users continue to experience significant difficulty understanding speech. One potential issue is that neurons in the auditory cortex do not respond to ABI stimulation in the same way as they respond to auditory stimulation. To study this issue, we used a novel chronic ABI model in mouse, allowing us to directly compare neural activation in the contralateral auditory cortex (ACtx) in response to sound and to electrical ABI stimulation.

The model used a flexible three-electrode ABI array to electrically stimulate the dorsal subdivision of the cochlear nucleus (DCN) in awake mice. The strain of mice used in this study express a genetically-encoded calcium indicator in cortical excitatory pyramidal neurons (Thy1-GCaMP6s or VGlut1-GCaMP8s). Two-photon imaging was performed through a cranial window over ACtx to measure sound and ABI electrically-evoked responses at both the single-neuron and populational levels. Neurons studied were within L2/3 (~250µm depth) of ACtx. Responses to amplitude modulated (AM) sounds and ABI stimulation were compared across a range of modulation depths.

ABI stimuli elicited diverse calcium responses in ACtx, including neurons with rapid transient activation, delayed sustained responses and stimulus-evoked suppression – response profiles

broadly similar to those evoked by sound. Approximately 30% of neurons responded to ABI and a similar proportion to sound stimuli, however only a small fraction responded to both, suggesting largely non-overlapping populations. Although many neurons displayed sharp tuning to specific modulations in acoustic and electrical stimuli, tuning preferences for AM-ABI and AM-sound were often mismatched at the single-neuron level. These findings suggest that although the ACtx is capable of decoding ABI inputs, these signals may not be immediately aligned with natural auditory coding strategies. This mismatch may contribute to the limited perceptual performance of ABI users and highlights the potential for cortical plasticity to enhance outcomes—aligning with clinical reports that ABI performance improves with continued device use. Ongoing work aims to determine whether behavioral training can reshape cortical tuning to ABI stimuli and enhance perceptual performance.

Podium 7: Neural Correlates of Tinnitus

3:00 p.m. - 5:00 p.m.

Meeting Room 208ABC, Floor 2

Moderators: Joel Berger and Nour Alsabbagh

Integrating Afferent and Efferent Auditory Assessments to Explore Central Gain and Hidden Hearing Loss in Tinnitus

3:00 p.m. - 3:15 p.m.

Pauline Devolder^{*1}, Hannah Keppler², Sarah Verhulst¹

¹*Hearing Technology at WAVES, Ghent University*, ²*Ghent University, Ghent, Belgium*

Background: Tinnitus is a symptom that is often associated with aging or excessive noise exposure. Emerging evidence suggests that interactions between peripheral hearing loss and central gain changes can alter central auditory and non-auditory regions, potentially contributing to tinnitus. Peripheral and central imbalances may affect both upward neural encoding and downward feedback pathways. This study combines novel afferent and efferent auditory measurements to examine how these factors interact and shape auditory encoding in individuals with tinnitus.

Methods: To assess afferent neural encoding from the cochlea to the inferior colliculus, the protocol included auditory brainstem responses (ABRs), envelope-following responses (EFRs), and frequency-following responses (FFRs) to low-frequency harmonic complex tones and speech syllables. Low-frequency FFRs allow us to differentiate between envelope and temporal fine structure encoding. The efferent reflex pathways were assessed using wideband click-evoked middle-ear muscle reflexes (MEMR) and wideband medial olivocochlear reflexes (MOCR). Additionally, several behavioural and tinnitus-related measurements were implemented. 120 participants were included: sixty with chronic tinnitus (noise-induced or idiopathic) and sixty age-matched controls, subdivided into young (18 – 35 y/o) normal-hearing, older (45 – 65 y/o) normal-hearing, and older hearing-impaired groups. Group comparisons evaluated the effects of tinnitus, age, and hearing loss on auditory processing.

Results: Participants with tinnitus had enhanced neural envelope encoding and improved behavioural low-frequency vowel discrimination. While typical listeners, as reported in previous studies, usually rely on envelope information to encode speech in noise, participants with tinnitus showed a correlation between neural temporal fine structure encoding and low-pass speech-in-noise scores. As expected, older listeners showed reduced responses on almost all measurements, which interacted with tinnitus-related enhancements. MEMR analyses revealed significant age-related effects but no direct tinnitus-related differences. This suggests that efferent function alone is insufficient to explain tinnitus-related enhancements and central gain.

Conclusions: These findings highlight the importance of considering interactions between peripheral damage, aging, and central gain in tinnitus research. By integrating afferent and efferent measures, this study establishes a comprehensive framework for interpreting changes in subcortical auditory encoding related to tinnitus. This framework also paves the way for future research on central gain in relation to aging, tinnitus, and hyperacusis.

Work supported by FWO G068621N AuDiMod.

Neural Plasticity Following Restricted Peripheral Cochlear Deafferentation in Ferrets

3:15 p.m. - 3:30 p.m.

Victoria Bajo*¹, Joshua R. Gold¹, Sara Rolle Sonora¹, David N. Furness², Linus Milinski¹, Andrew J King¹, Fernando R. Nodal¹

¹*University of Oxford*, ²*Keele University*

Background: Subjective tinnitus is the perception of a sound in the absence of an environmental source, and is thought to derive from maladaptive plasticity following hearing impairment. Our aim was to investigate whether primary auditory cortex has a causal role in a model of trauma-induced tinnitus.

Methods: Our longitudinal experimental design comprised gap-in-noise behavior using positive operant conditioning and auditory brainstem response (ABR) recordings to detect the presence of tinnitus before and after making a restricted unilateral mechanical lesion in the cochlea. Normal hearing ferrets underwent behavioral testing on a two-alternative forced choice paradigm and ABR measurement prior to spiral ganglion lesion. Post-lesion, the animals were re-tested behaviorally and ABRs were recorded at two time points (1 week and 3 months). Animals were tested again in the gap-in-noise task after deficient replicant AAV virus containing ArchT tagged with GFP was injected bilaterally in the primary auditory cortex (A1) and 200 μ m optical fibres were implanted at the surface of A1 to silence cortical activity. Pulses of green light ($\lambda = 532$ nm, 8-10 mW) from a compact diode-pumped solid-state (DPSS) laser were randomly paired with the sound stimuli and applied to A1 on the right, the left or both sides of the brain in 50% of the trials presented. To investigate neuroplasticity in A1 neurons, recordings were performed using Neuronexus probes in the two hemispheres and compared with recordings from naïve animals.

Results: Spiral ganglion lesion resulted in a heterogeneous change in gap-in-noise detection behavior across animals, with some ferrets displaying temporal processing impairments and

others showing post-lesion adaptation. These behavioral outcomes correlated with particular ABR features, indicating a possible predisposition towards adaptation/maladaptation. Interestingly, optogenetic silencing of A1 contralateral to the lesioned cochlea showed a rescue effect on the behavioral markers of impairment.

Spiral ganglion lesions appear to reduce the afferent drive to the A1 with recorded units showing lower spontaneous firing rates than in naïve controls. Importantly, when behavioral status was considered, the spontaneous firing rate of A1 neurons from cases that did not show a behavioral impairment was significantly lower and more synchronized than that in animals that showed a behavioural impairment. Frequency remapping in A1 was not observed following spiral ganglion lesions, though in control cases, the right hemisphere showed a greater representation of low frequencies than the left hemisphere, whereas this inter-hemispheric asymmetry was not observed in the lesioned cases, where A1 units were significantly more narrowly tuned than those in control animals.

Conclusions: These results suggest that A1 shows evidence for behaviorally-relevant deafferentation-related neuroplasticity, suggesting possible targeted strategies for remediation of the maladaptive processing potentially underpinning tinnitus in humans.

Development of a New Tinnitus Model Using Designer Receptors Exclusively Activated by Designer Drugs (DREADD)-Based Chemogenetic Technology

3:30 p.m. - 3:45 p.m.

Joseph Pinkl^{*1}, Giovana Da Silva², Harry Wang², Jianxin Bao¹

¹Gateway Biotechnology Inc., ²Duke University

Background: Tinnitus research at the preclinical stages is difficult to manage due to a lack of understanding of its neurological mechanisms and limited reliable tests to identify tinnitus in animal models. Evidence obtained from both animal and human studies suggest that tinnitus results from the dysfunction of several neuronal networks. Here, we aimed to develop a new pathological model of tinnitus by directly modulating neuronal activity of the inferior colliculus (IC) in mice using Designer Receptors Exclusively Activated by Designer Drugs (DREADD)-based chemogenetic tools, a class of engineered proteins that are activated by synthetic ligands. Validation measures were completed using a behavioral-based tinnitus detection method called sound-based avoidance detection (SBAD). Additionally, we supplement SBAD data with deep learning video tracking analyses to profile tinnitus-associated passive behavior.

Methods: Eight adult C57BL/6J mice received bilateral stereotaxic micro-injections of AAV8-CaMKIIa-hM3D(Gq)-mCherry (AAV) at 50-150 nL doses in the central nucleus of the IC (AP: -5.00 mm, ML: +/- 1.00 mm, DV: -1.50 mm). After one month of recovery, animals underwent 15 days of SBAD training followed by 6 consecutive days of SBAD testing. On every other day of testing, animals received intraperitoneal (i.p.) injections of clozapine-N-oxide dihydrochloride (CNO), a chemogenetic actuator, at a dose of 5 mg/kg, one hour before testing. On all other days of testing, animals received .5ml injections of .9% saline as a control. Video tracking was completed using an overhead camera capturing animal movement within a 15inx8inx5in cage. Video recordings were obtained from each animal individually under two conditions – 1 hour post i.p. CNO injections and 1 hour post i.p. saline injections.

Results: For AAV injected animals, there was a significant main effect of injectant type (saline vs CNO) for the SBAD silent trials only. However, there was no significant main effect of AAV dose and no significant interaction between injectant type and AAV dose for both silent and sound trials. Silent trial scores post-CNO injection ranged from approximately 50-100% with seven of the eight IC AAV injected animals yielding scores that indicated tinnitus-associated behavior. Video tracking analyses indicated 9 distinct metrics of animal behavior that yielded significant differences between injectant type (saline vs CNO).

Conclusions: SBAD scores suggest that selective modulation of IC excitatory neurons via chemically activated DREADDs can lead to tinnitus-associated behavior. Video tracking analyses identified 9 behavioral metrics that may be associated with tinnitus percept. Future studies will continue to build these tinnitus-associated behavioral profiles. Because IC hyperactivity is implicated in many tinnitus studies, excitatory DREADD applications in auditory pathways could be reliable experimental tools for tinnitus research.

Identify Tinnitus-Related Neural Activity With a Machine Learning Classification Model

3:45 p.m. - 4:00 p.m.

Jinsheng Zhang^{*1}, Na Zhu², Hao Luo³

¹Wayne State University, ²University of Michigan, ³Henry Ford Health

Background: Tinnitus is a prevalent medical condition that affects many millions of people. Efforts have been made to better understand its underlying mechanisms and develop treatment. However, there is a lack of reliable biomarker for diagnosing tinnitus. In animal studies, it has been shown that behavioral evidence of tinnitus is associated with hyperactivity often in the forms of higher firing rate. However, there is a significant difference among individuals. That is, firing rates alone may not be the only factor in determining the presence or absence of tinnitus. In this study, we adopted an integrated approach, leveraging machine learning (ML) to examine spike rates and spike patterns, in order to separate animals with and without tinnitus. Ultimately, our goal is to develop a ML tool that can reliably identify biomarkers for tinnitus by evaluating waveform measurements from neural activity.

Methods: In this study, spontaneous activity was recorded in the auditory cortex (AC) of seven rats with known tinnitus status using gap prepulse inhibition of the acoustic startle paradigm: four displaying behavioral evidence of tinnitus, and three without tinnitus. During the signal processing, local field potentials were analyzed to determine the mean and standard deviation, providing data's values and range. Statistical measures were used to detect spikes in the time domain. A short segment of the waveform was extracted at each spike location, ensuring the segment capturing the entire spike waveforms. A total of 10,000 spike samples were used to train and test a supervised ML model.

For the ML process, a model was developed using four input features: spike firing rate, spike amplitude, prominence, and width. The output prediction was a binary value: "0" indicating no tinnitus, and "1" indicating tinnitus was observed. Seventy-five percent of the data were used for model training, while the remaining 25% was used for validation. The ML algorithms included

decision trees and support vector machines (SVM), and their validation accuracy was compared. The importance of the four features in model building was assessed using various ranking algorithms, such as Minimum Redundancy Maximum Relevance (mRMR) and the Chi-squared test.

Results: Our results demonstrated that the ML can identify the presence of tinnitus with an accuracy of approximately 90% in several methods, including the SVM, k-nearest neighbors (KNN), and tree-based models. Among these, the bagged tree algorithm demonstrated the highest accuracy. Additionally, the firing rate, spike amplitude, and prominence are the three most important input features.

Conclusions: The machine learning model may be further developed to an effective tool for identifying a biomarker of tinnitus.

Neuroinflammation of Central Auditory and Limbic System Induced by Blast Exposure

4:00 p.m. - 4:15 p.m.

Wei Sun^{*1}, Li Li¹, Guangdi Chen¹

¹*University at Buffalo*

Background: Tinnitus and memory deficits are commonly seen in veterans who have traumatic brain injury (TBI) during their service. There is supporting evidence suggesting that blast-caused neuroinflammation may underline these symptoms.

Methods: Two-choice paradigms of the operant conditioning tests were used to test tinnitus and hyperacusis in adult Sprague Dawley rats induced by blast exposure. Immunocytochemistry was used to detect brain inflammation signals in the central auditory system and hippocampus using microglial cell markers. To isolate blast induced brain injury affected by peripheral auditory damage, the ear canals of the rats were plugged during the blast exposure. Rats without blast exposure were used as controls.

Results: Rats in the TBI group with ear protection did not develop hearing loss. The reaction time showed a significant decrease, indicating increased sound sensitivity, in blast exposed rats with ear protection. In the TBI groups, increased neural inflammation expression of IBA-1 and CD-68 signals were detected in both the hippocampus and auditory pathways, compared to controls.

Conclusions: Our data found blast-induced neuroinflammation in the central auditory system and hippocampus area may be linked to tinnitus and memory deficits after blast exposure.

Sound-Evoked Pain Behavior Analyzed by Machine Learning Exceeds a Threshold Set by a Known Pain Model

4:15 p.m. - 4:30 p.m.

Benjamin Seicol^{*1}, Amelie Valles², Bryan Djunaidy², Elisabeth Glowatzki¹, Megan Wood³

¹*Johns Hopkins School of Medicine*, ²*Johns Hopkins University*, ³*Vanderbilt University School of Medicine*

Background: The auditory pain threshold in humans is commonly reported to be ~120 dB (Sherlock and Formby, 2005). This threshold was thought to be similar in mice, and loud sound exposure has been routinely employed for noise-induced hearing loss in rodent models. To investigate whether high-intensity sound evokes behaviors that indicate pain, calcitonin gene-related peptide (CGRP) was used to induce migraine-like pain as a comparative tool to validate our sound-evoked behavior assay. CGRP was selected as the painful stimulus of interest due to its known expression in the auditory system (Cabanillas and Luebke, 2002; Schrott-Fischer et al., 2005), its ability to lower auditory thresholds (Rahman et al., 2025; Le Prell et al., 2021), and its association with phonophobia (heightened auditory sensitivity or pain), a symptom frequently reported by migraine sufferers (Lipton et al., 2024).

Methods: C57BL/6J mice aged 6 – 10 weeks old were recorded inside a custom-built clear container in a sound chamber. CGRP protocol: Mice were first recorded for 10 minutes to establish baseline behavior. Behavioral recordings were conducted following CGRP injection (100 µg/kg, i.p.) or saline during the middle of the migraine period (20–40 minutes post-injection), which included the expected peak at approximately 30 minutes post-injection (Rea et al., 2018). Sound protocol: Mice were recorded for 10 minutes to establish a baseline and then exposed to 2 – 20 kHz sound for 2 minutes ranging from 70 – 120 dB SPL interleaved with 2 minutes of no sound. Deep learning was applied to automatically place thirteen pose-estimated points and connecting skeletons on the face and body of the mice. Six facial grimace measurements (La Moëne and Larson, 2023) and four new body positions parameters were separately added together to calculate two cumulative scores.

Results: After CGRP injection, both Facial Grimace and Body Position Scores were statistically distinct from baseline recordings, indicating pain. Saline-injected controls did not show a significant change in Facial Grimace or Body Position Scores. Pain thresholds for sound were similarly defined by a significant difference from baseline in both additive Facial Grimace and Body Position Scores. Normal hearing mice were found to have auditory pain thresholds of 100 dB SPL based on these criteria that also exceeded the magnitude of change in CGRP-injected mice. Animals returned to baseline behavioral levels during periods of no sound following sound presentations, except for post 120 dB SPL-exposure.

Conclusions: This study highlights the need for caution when exposing animals to sound levels above 100 dB without appropriate anesthesia or analgesia to mitigate potential pain. Machine learning analysis of video recorded mice during sound presentation proved to be an effective approach to measuring auditory pain in mice and could be applied to hyperacusis models.

Use of Opm-Meg for Identification of the Neural Correlate of Tinnitus

4:30 p.m. - 4:45 p.m.

Marlies Knipper^{*1}, Rodrigo Donoso-San Martín², Stephan M. Wolpert³, Stefan Fink³, Paul Delano², Christoph Braun⁴, Lukas Rüttiger³

¹*University Hospital Tuebingen*, ²*Laboratorio Neurobiología de la Audición, Facultad de Medicina, Universidad de Chile*, ³*Tübingen Hearing Research Centre, University of Tübingen*,

⁴*MEG-Center, University of Tübingen, Otfried-Müller-Straße. and Hertie Institute for Clinical Brain Research*

Background: Acquired auditory processing disorders including age dependent hearing loss, speech discrimination deficits, tinnitus or hyperacusis, require a personalized diagnosis to assign the individual cause within the auditory hierarchy to either the periphery, subcortical, or distinct cortical or cortico-fugal neuronal dysfunctions. The good functioning of the feedforward and feedback PV-IN network is an essential precondition for audition that above all senses relies on high-speed information flow (Zajac IT and Nettelbeck T, 2018). Therefore, we hypothesize disease-specific deficits in temporal intracortical network function in auditory circuits.

Methods: We studied fast auditory processing in tinnitus subjects with or without the comorbidity of hyperacusis using auditory stimuli evoking time-sensitive cortical responses. These responses were recorded using a time-sensitive OPM-MEG.

Results: We expect this method to become an efficient diagnostic strategy to fathom peripheral or central contribution of the distinct auditory impairments in the future to improve individualized targeted interventional therapies.

Conclusions: Here we will present preliminary results demonstrating the usability and function of the OPM-MEG for hearing research in a clinical setting.

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Permanent Reversal of Noise-Induced Tinnitus by 28-Days of Ebselen Treatment is Related to Significant Reductions in Outer Hair Cell Loss

4:45 p.m. - 5:00 p.m.

Kushal Sharma¹, Annie Jia¹, Rende Gu¹, Sao-Mai Gahr¹, Ryan Longenecker¹, Jonathan Kil*¹

¹*Sound Pharmaceuticals, Inc.*

Background: Noise-induced hearing loss (NIHL) is a major risk factor for developing noise-induced tinnitus (NIT). Prior human studies have shown that 4-days of ebselen can prevent acute NIHL in young adults and 21-28 days of ebselen can improve hearing and tinnitus in aged adults with Meniere's disease. We previously showed in young and old mice that 4 or 14-days of ebselen treatment can temporarily reduce or partially reverse NIT. The goal of this current study was to determine if 28-days of ebselen treatment can permanently reverse NIT in young (3 to 4-month) and old mice (12 to 15-month) and if it is related to outer hair cell (OHC) loss.

Methods: Two ages of CBA/CaJ mice (N=60) were utilized: 3 to 4-month-old (N=30) and 12 to 15-month-old (N=30). Each age group was further divided: Group 1: 3-6 month (N=6) and 12-15 month (N=6) served as an unexposed controls with no ear occlusion; Group 2: 3-4 month (N=24) and 12-15 months (N=24) received a temporary unilateral occlusion of an external ear canal before a brief narrowband noise exposure (awake, 112 dB, two hours, octave-band noise centered at 8-kHz). Baseline ABRs and Gap-Induced-Prepulse-Inhibition of the Acoustic Startle Reflex (GPIAS) were assessed prior to and three months after noise to determine changes in

GPIAS ratios (behavioral marker for NIT). The mice that developed NIT were treated with 28-days of ebselen (10/mg/kg/d/ip) and reassessed at 1, 10, and 30-days post-treatment. Cochlear histopathology was performed using Myosin-7a immunolabeling to identify OHC loss.

Results: Three months after noise exposure, permanent threshold shifts were observed in the open ears of all noise-exposed mice across three tested frequencies (16-32 kHz). GPIAS showed frequency specific changes (NIT) in a subset (N=13) of the noise-exposed mice (N=48). After 28-days of ebselen, gap detection deficits were significantly reduced to pre-exposure levels in 12 of the 13 (92%) NIT mice at 1-day, in 10 of 12 (83%) NIT mice at 10-days, and in 9 of 11 (82%) NIT mice at 30-days post-treatment. Mice with permanent reversal of NIT showed significantly less OHC loss (3 complete rows) than those with temporary reversal: 0.073 ± 0.07 mm vs 0.660 ± 0.06 mm, respectively ($p=0.0013$).

Conclusions: 28-days of ebselen treatment can permanently reverse gap detection deficits to baseline or pre-noise exposure in 82% of mice that developed NIT, 3-months after noise exposure and the development of NIHL. In addition, the significant reduction in complete OHC loss suggests a broad therapeutic window by which to prevent injured OHCs from dying. This work is the first demonstration of an anti-inflammatory drug permanently reversing NIT after the development of age-related hearing loss. These data further support ebselen as a treatment for NIHL and NIT in young and old adults.

gEAR Session: Advanced Workshop

4:15 p.m. - 5:15 p.m.

Meeting Room 209ABC, Floor 2

Join us for an exciting workshop where you'll learn directly from leading investigators how to harness the power of the **gEAR platform** to explore cutting-edge, newly generated **multi-omic datasets** in our field—and accelerate your own research. We'll kick off with a brief introduction from the gEAR team, followed by three, brief, expert-led sessions:

- **Brian Herb** - Showcasing the **Hearing Restoration Project's new collection** of multi-omic datasets now available in gEAR. Brian will demonstrate how to use the **gEAR projection tool** for transfer learning and for identifying shared molecular features across diverse datasets.
- **Matthew Kelley** - Presenting the **first release of CoMAP**, the NIDCD's cochlear multi-omic and spatial transcriptomic atlas. Matthew will guide you through co-exploring **transcriptomic and spatial transcriptomic data** using gEAR's integrated tools.
- **Celia Bloom** - Sharing a **new gene regulatory network** from the lab of Litao Tao and demonstrating how to use the **new epigenomic viewer** in gEAR. Celia will walk through how to visualize and connect **DNA accessibility, epigenetic marks, CUT&RUN, and Hi-C data** with transcriptomic datasets—all within the platform.

Advanced gEAR Workshop

Brian Herb, *University of Maryland School of Medicine*

Presenting the First Release of CoMAP, the Nidcd's Cochlear Multi-Omic and Spatial Transcriptomic Atlas. Matthew Will Guide You through Co-Exploring Transcriptomic and Spatial Transcriptomic Data Using Gear's Integrated Tools

Matthew Kelley, *NIH/NIDCD*

Sharing a New Gene Regulatory Network from the Lab of Litao Tao and Demonstrating How to Use the New Epigenomic Viewer in Gear. Celia Will Walk Through How to Visualize and Connect Dna accessibility, Epigenetic marks, CUT&RUN, and Hi-C Data with Transcriptomic datasets—all within the Platform.

Celia Bloom, *Creighton University*

Inner Ear Courses

6:30 p.m. - 7:30 p.m.

Meeting Room 208ABC, Floor 2

Transforming Vestibular Care Through Clinical Training: A Framework from the EU MSCA PROVIDE Doctoral Network and the Bárány Society's Vestibular Medicine Program

Sonja Pyott, *Carl von Ossietzky University of Oldenburg*

Transforming Vestibular Care through Clinical Training: A Framework from the Eu Msca Provide Doctoral Network and the Bárány Society's Vestibular Medicine Program

Raymond van de Berg, *Maastricht University Medical Center+*

JAX Course

Matthew Kelley, *NIH/NIDCD*

Inner Ear Courses: NIDCD EARssentials Course

Elyssa Monzack, *NIDCD*

Biology of the Inner Ear

Katie Kindt, *NIH/NIDCD*

NIDCD Workshop: Changes at the NIH for Established Investigators

6:30 p.m. - 8:00 p.m.

Meeting Room 209C, Floor 2

Over the past year, the NIH has introduced several significant policy changes that directly affect current NIDCD awardees. This workshop is designed to provide clear guidance on these updates and equip awardees with the knowledge and strategies needed to successfully navigate the evolving requirements.

Changes at the NIH for Established Investigators

Meeting Room 209C, Floor 2

Janet Cyr, *NIH/NIDCD*

Samantha Tempchin, *NIH/NIDCD*

Becky Wagenaar-Miller, *NIH/NIDCD*

SEED.NIH.GOV Helping Innovators Turn Discovery Into Health

6:30 p.m. - 8:00 p.m.

Ballroom B, Floor 3

SEED grants are made to U.S.-based companies with fewer than 500 employees and have been the largest source of early-stage capital for life sciences in the U.S., specifically to support research and development within small businesses. The grant program expired on Sept 31, 2025, so the workshop will begin with an update on its current status.

Seed.NIH.GOV Helping Innovators Turn Discovery Into Health

Ballroom B, Floor 3

Roger Miller, *NIDCD/NIH*

Tuesday, February 10, 2026

Podium 14: Inner Ear: Cochlear Mechanics

8:00 a.m. - 10:00 a.m.

Meeting Room 208ABC, Floor 2

Moderators: Hamid Motallebzadeh and Jong-Hoon Nam

Cochlear 3D Pressure Focusing and Cochlear Amplification

8:00 a.m. - 8:15 a.m.

Alessandro Altoe*¹

¹*University of Southern California, Caruso*

Background: It has long been known that the three-dimensional geometry of the cochlea makes an important contribution to cochlear vibration, especially when waves are short near their characteristic place. However, the literature is unclear regarding the magnitude of this contribution: depending on the assumptions made, hydrodynamic effects and their dependence on cochlear geometry are often down-played or over-emphasized. In addition, descriptions of these hydrodynamic effects are very technical, making it hard to estimate the contribution of hydrodynamics to cochlear mechanical data.

This work elucidates how 3-D cochlear hydrodynamics produce “pressure focusing”—i.e., how much the 3-D hydrodynamics boost cochlear vibrations—and how this effect depends on cochlear geometry and wavelength. In particular, our work revisits classic simplifying assumptions regarding cochlear wave propagation, examines their impact on the estimation of pressure focusing, and consequently estimates the relevance of pressure focusing for the operation of the real cochlea.

Methods: We extend the 2-D “pressure focusing” framework developed by Duifhuis to study 3-D wave propagation. This framework employs the same equations used in all other models and classic studies, but it has two main advantages: i) allows us to estimate directly the contribution of hydrodynamic effects to BM vibration; ii) it enables the construction of highly controlled models whose behavior depends on measured properties of cochlear wave propagation rather than on delicate assumptions. We incorporate our findings into an active model of the gerbil cochlea that includes realistic wavelengths and cochlear dimensions.

Results: Our results highlight that, regardless of various possible simplification, the pressure-focusing effect is necessarily large in the base of the cochlea, where waves are short, the duct is tall, and the BM is narrow.

This effect is particularly prominent in the active cochlea, where waves are shorter than in the post-mortem condition. In the base of the cochlea, amplification produces an increase in near-CF BM vibration—relative to the post-mortem state—of about 40 dB. Our results further indicate that, given cochlear dimensions and measured wavelength, roughly 20 dB of this increase is caused by “pressure focusing”.

Conclusions: This work extends our previous findings regarding the importance of geometry for cochlear responses while making it easier to estimate hydrodynamic effects from the data. Most importantly for the ARO audience, it demonstrates that one can estimate, rather simply and reliably, the magnitude of hydrodynamic effects on cochlear vibration. Estimates using available data from the gerbil reveal that pressure focusing is especially prominent at the base of the cochlea, acting as a facilitator of high-frequency amplification. Pressure focusing is a major player in active amplification and must be accounted for correctly to understand how the cochlea works.

Marrying Critical Oscillators With Traveling Waves Shapes Nonlinear Sound Processing in the Cochlea

8:15 a.m. - 8:30 a.m.

Henri Ver Hults¹, Carles Blanch Mercader¹, Frank Jülicher², Pascal Martin*¹

¹CNRS / Institut Curie-PSL University / Sorbonne University, ²Max Planck Institute for the Physics of Complex Systems / Cluster of Excellence Physics of Life, Technische Universität

Background: The cochlea's capacity to process a broad range of sound intensities has been linked to nonlinear amplification by "critical oscillators", where each cross-section of the organ of Corti operates near an oscillatory instability called a Hopf bifurcation. However, while the increasing sensitivity of a critical oscillator upon decreasing the stimulus magnitude comes with proportionally sharper frequency tuning and slower responsiveness—critical slowing down, the bandwidth of cochlear frequency tuning and the response time vary little with level. In this work, we address this qualitative discrepancy by performing a systematic study of traveling-wave models based on critical oscillators. We show that the cochlear response to sound cannot be reduced to that of a critical oscillator in isolation and clarify how cochlear tuning curves are shaped by a complex interplay between local mechanical properties and distributed effects from traveling waves.

Methods: We developed a nonlinear box model of the cochlea in which the organ of Corti was represented by a string of tonotopically-distributed critical oscillators. Their equation of motion obeyed a generic nonlinear equation, called the normal form, of a complex variable whose real part represents the transverse motion of the basilar membrane. We examined how frequency-tuning curves were influenced by two-dimensional hydrodynamics, mechanical coupling between the oscillators, and energy pumping into the traveling wave as a function of the sound-pressure level. We confronted time-domain simulations of the model to published measurements of basilar-membrane vibrations in the chinchilla cochlea at a fixed position of characteristic frequency $CF = 6.6$ kHz.

Results: We found that the model can produce, with a single set of parameters, a family of cochlear tuning curves that quantitatively describe experimental data over a broad range of input levels. Importantly, mechanical coupling between the oscillators and energy pumping were both essential to decouple sensitivity and bandwidth, which were otherwise dictated solely by local resonance and, thus, inversely related. Coupling limited the peak sensitivity at low sound-pressure levels, increased the bandwidth and controlled the drop in sensitivity beyond the characteristic frequency. A traveling wave riding on a tonotopic organization of oscillators with descending natural frequencies automatically benefitted from accumulation of energy gain,

which could reach high levels away from the resonant place. Finally, by accounting for the complex nonlinear behavior of cochlear mechanics, the interplay between the generic nonlinear behavior of critical oscillators—the only nonlinearity in the model—and traveling waves captured the essence of the cochlear nonlinearity.

Conclusions: Maintaining relatively broad and level-independent tuning curves while amplifying weak sound stimuli is essential to endow the cochlea with a broad dynamical range of responsiveness and a relatively fast responsiveness to weak stimuli. Marrying tonotopically distributed critical oscillators with traveling waves provides a fundamental mechanism to explain this endeavor.

Level Dependence of Asymmetries in Cochlear Suppression by Upward and Downward Frequency Sweeps

8:30 a.m. - 8:45 a.m.

Karolina Charaziak*¹

¹*USC Keck School of Medicine*

Background: The acoustic environment is often a complex mixture of competing sounds that, through interactions within the auditory system, can mask or reduce the audibility of target signals. Masking by stationary sounds, such as pure tones, is relatively well studied, but the mechanisms underlying masking by dynamic sounds that change over time remain less understood. For example, upward frequency sweeps, in which the instantaneous frequency increases over time, are typically more effective at masking tonal and speech signals than their time-reversed copies (downward sweeps). This sensitivity of masking to sweep direction is thought to originate in the cochlea, where traveling-wave dispersion can be either enhanced or counteracted by the sweep depending on direction of frequency change. However, whereas the rate of cochlear dispersion varies relatively little with stimulus intensity, behavioral data indicate that masking sensitivity to sweep direction decreases as stimulus level increases. Here, we test whether interactions between intensity-dependent cochlear suppression (a known contributor to masking) and intensity-invariant cochlear dispersion can account for these behavioral observations.

Methods: We measure suppression as seen directly in vibrations of basilar membrane at the apical turn of the mouse cochlea (tuned to ~9 kHz) using optical coherence tomography. Suppression sensitivity to sweep direction was defined as the difference in suppression of a probe tone response produced by upward versus downward sweeps of equal intensity. Signal-processing techniques were applied to extract iso-intensity suppression tuning curves as a function of instantaneous sweep frequency, corrected for cochlear delays.

Results: When the sweep rate was adjusted to approximate the natural dispersion rate of the mouse cochlea, upward sweeps produced stronger suppression of probe responses than downward sweeps. Sensitivity to sweep direction decreased with increasing suppressor intensity but was unaffected by changes in the probe-tone intensity. The iso-intensity suppression tuning curves showed that the low-frequency side broadened significantly as sweep level increased, independent of sweep direction. In contrast, suppression on the high-frequency side was largely intensity-invariant but strongly modulated by sweep direction, with broader tuning produced by upward vs downward sweeps.

Conclusions: As the sweep rate approached the natural cochlear dispersion rate, upward sweeps became more effective suppressors than downward sweeps, consistent with human masking data. Although overall suppression increased with sweep intensity, its sensitivity to sweep direction was most pronounced at lower intensities. The reduction in sensitivity to sweep direction at higher intensities can be explained by changes in suppression tuning: low-frequency suppression is independent of sweep direction but increases substantially with intensity due to the spread of excitation, whereas high-frequency suppression remains intensity-invariant yet direction-sensitive. Thus, the decrease in sensitivity to sweep direction at higher levels arises from a relative increase in low-frequency suppression rather than a change in the underlying mechanism of upward versus downward sweep suppression.

Level Effects on Two-Dimensional Motion Across the Three Rows of OHCs in the Mouse Apex

8:45 a.m. - 9:00 a.m.

Gabriel Alberts^{*1}, Wiam Lahlou², Sunil Puria³

¹*Harvard University*, ²*International University of Rabat*, ³*Harvard Medical School, Mass. Eye and Ear*

Background: Optical coherence tomography (OCT) measurements, pioneered for hearing research by this year's Award of Merit recipient Alfred Nuttall (Wang and Nuttall, 2010), have greatly expanded our understanding of cochlear micromechanics. Recent studies revealed sharper tuning and a larger dynamic range in the tectorial membrane compared to the basilar membrane (Lee et al., 2015), a “hotspot” of motion around outer hair cells (OHCs) in the center of the organ of Corti (Cooper et al., 2018), and flexibility between rows of OHCs around their tops in the reticular lamina (Cho and Puria, 2022). While these investigations revealed many aspects of the mammalian cochlea in vivo, it remains unclear why the canonical three rows are more conducive for cochlear amplification than a single row. Here, we measured two-dimensional motion in the mouse cochlear apex to investigate how motion across the rows correlated with level and provide insights on the nature of organ of Corti mechanics.

Methods: Vibration measurements were acquired using a 905nm wavelength Thorlabs OCT system, which has a higher axial resolution than other systems in use allowing us to compare motion across OHC rows. The OCT head was mounted onto a six-axis robot arm which supported our two-dimensional measurements in the radial-transverse plane. The orientation and angle of the organ of Corti was quantified using the robot coordinates and volumetric orientation method described in Frost et al. (2022, 2023). Measurements were made using pressure-equalized tone sweeps presented in the ear canal. The stimulus and organ of Corti vibrations were captured using in-house LabView programs SyncAv and VibOCT, respectively. Postprocessing was performed in MATLAB.

Results: Preliminary results indicated that the first row of OHCs exhibited more level compression and moved more at lower levels than the other rows, but this did not extend to the adjacent outer pillar cell. The effects were seen across frequencies and were more prominent below the best frequency. Radial and transverse motions were broadly similar across levels and resulted in mostly spatially lineal motion along the axis of the OHCs. The first row showed a

slight phase lead compared to the second and third rows at higher levels and became more in phase at lower levels.

Conclusions: By integrating our high-resolution OCT system and robotic arm, we were able to measure two-dimensional motion across the tops of the three rows of OHCs in the mouse apex. The data provides further evidence that the reticular lamina, comprised partly by the tops of OHCs, is flexible. Furthermore, the nature of the flexion changed with level, so the variation between rows may play an important role in cochlear amplification. [Supported by the grants R01DC07910, F31DC021079, and T32 DC000038 from the NIDCD of NIH.]

Motion in the Lateral Region of the Organ of Corti Suggests “Fluid-Like” Properties Contribute to Traveling Wave Damping

9:00 a.m. - 9:15 a.m.

Lauren Chiriboga^{*1}, C. Elliott Strimbu¹, Elizabeth S. Olson¹

¹*Columbia University*

Background: The passive mechanics of the organ of Corti (OC) are the foundation of sound transduction of the hearing system. The interaction of the OC and the scala fluid provides the passive basis of the traveling wave. One aspect of traveling wave mechanics that is not fully understood is what causes the traveling wave to lose amplitude at the correct frequency-dependent place. It has been noted by models and experiments that viscous forces induced by fluid velocity gradients would contribute to traveling wave damping. We examine fluid-like velocity gradients in the lateral supporting cells of the guinea pig via OCT motion measurements.

Methods: We performed intra-OC vibrometry to measure motion in a transverse view in the base of the guinea pig OC. Our measurements covered the lateral region of the OC, including measurement locations at the basilar membrane (BM), Hensen’s cells, Claudius’ cells and Boettcher’s cells. We monitored cochlear condition by measuring distortion product otoacoustic emissions. We compared velocity gradients found via motion measurements to those predicted by a 3-D cochlear model by first calculating the fluid velocity potential (Steele and Taber, 1979). The Steele and Taber model uses the WKB approximation and incorporates both fluid mechanics and organ of Corti mechanics. We visualize the fluid velocity and velocity potential as both a function of wavenumber (k) and distance from the BM (z).

Results: We examined motion throughout the lateral supporting cells as distance increased from the BM. We observed motion decay as distance from the BM increased. We also observed a layer adjacent to the BM, spanning approximately 10-20 microns, in which motion amplitude remained constant. This corresponded to a bright region in the lateral supporting cells that we have attributed to the Boettcher’s cells. Beyond this layer, the velocity decay with distance in the cells of the OC was similar to what is predicted to exist in the scala fluid adjacent to the BM by the Steele and Taber model.

Conclusions: Given the similarity of the lateral region motion data and fluid decay predicted by the model, the lateral supporting cells appear to behave like a fluid as opposed to as a solid mass. This is excluding the layer including the BM and Boettcher’s cells which moves uniformly. These fluid motion gradients could contribute to traveling wave damping by removing energy

from the system via viscous forces, causing the traveling wave to lose amplitude at the correct place for a given frequency.

Organ of Corti Vibrations in the Apex of the Gerbil Cochlea

9:15 a.m. - 9:30 a.m.

Sebastiaan Meenderink*¹, Wei Dong²

¹VA Long Beach Healthcare System, ²University of California, Irvine

Background: Low-frequency hearing is essential for speech perception, but the study of the involved (i.e., apical) cochlear mechanics remains lacking due to difficulties obtaining optical access to this region without compromising cochlear function. Optical coherence tomography (OCT) changed this. The goal of this research was to characterize sound-evoked vibrations in the organ of Corti (ooC) at the apex of the intact cochlea of the gerbil, an animal with a low frequency hearing range like that of humans.

Methods: Multi-tone stimuli (0.2–1.2 kHz; 30–70 dB SPL/component) and OCT were used to record sound-evoked vibrations from multiple longitudinal locations (per animal: 10–19 locations spanning 0.3–0.51 mm) in the third turn of gerbil cochleae (n=5, either sex, left ear). This yielded data from 74 distinct longitudinal locations. Depth-resolved phase of the reflected light was used to calculate the frequency specific amplitude and phase of the vibrations. These data were used to calculate metrics for tuning (e.g., Q6dB), group delay and wavelength.

Results: Analysis of 3rd-turn, basilar membrane (BM) vibrations showed that:

- o At 30 dB SPL, frequency response curves (FRC) were tuned. Tuning decreased with SPL and became low-pass-like at 70 dB SPL.
- o Best frequency (BF) at 30 dB SPL systematically decreased with distance from base, which was well described by the gerbil cochlea place-frequency map (Müller (1996), *Hear. Res.* 94:148–156). The observed BF's indicate that recording locations were close to (0.8–1.6 mm) the helicotrema.
- o Responses grew compressive at all test frequencies: at BF, amplitudes at 70 dB SPL exceeded 30 dB SPL responses by 5–25 dB (i.e., gain: 15–35 dB).
- o FRC often showed a supra-BF response plateau with group delays (GD) that were substantially shorter than for near-BF frequencies.
- o Phase at BF systematically increased with distance from base, GD did not. The latter showed a weak negative trend, but this was not significant.

More preliminary, the 3rd-turn data showed non-uniform vibration patterns across the ooC. For example, outer hair cell (OHC) responses at BF were ~0.5 cycle out-of-phase with the BM, although BM- and OHC-FRC were very similar in terms of BF, Q6dB and GD.

Conclusions: The tonotopic organization (i.e., a monotonically decreasing BF with distance from base) is maintained over the entire length of the cochlea. In addition, mechanical, sound-evoked responses in the apex of the gerbil cochlea are (broadly) nonlinear and have tuning characteristics comparable to those of low-BF auditory fibers in cranial nerve VIII.

We thank Ana K. Gallegos-Anchondo for help with data collection. This work was supported by NIDCD R21DC019998 and VA RRD Merit 1 I01 RX003491. These contents do not represent the views of the U.S. Department of Veterans Affairs or the United States Government.

Three-Row Mammalian Hair Bundle Model Predicts in Vitro Response Dynamics

9:30 a.m. - 9:45 a.m.

Varun Goyal^{*1}, Karl Grosh²

¹Stanford University, ²University of Michigan

Background: The mammalian outer hair cell (OHC) hair bundle (HB) responds differently to fluid-jet and probe stimulation in vitro, revealing different time scales in the mechanical and electrical responses depending on the configuration. Fluid-jet stimulation produces bundle creep with slow mechano-electrical transduction (MET) current adaptation. Under stiff probe stimulation, mechanical displacements are rapid, while both fast and slow current adaptation are seen. A mechanistic explanation for these differences, and the prevalence of three to four stereociliary rows in mammalian OHC HBs, is lacking.

Methods: We developed a nonlinear, viscoelastic, morphologically accurate three-row micromechanical model of a mammalian OHC bundle with two adaptive models representing the interaction of the three rows of stereocilia seen in most OHC HBs. A constrained version of the model was optimized to match experimental fluid-jet responses of a rat OHC HB at the 4 kHz best place (Caprara et al., Sci. Adv. 2020), then used to predict responses under stiff probe stimulation. We further analyzed activation curve widths, stiffness, and adaptive shifts under paired-pulse stimulation.

Results: The model reproduced fluid-jet responses, displaying a creep and slow adaptation, and probe responses, where fast adaptation was unmasked. Simulations showed that during fluid-jet excitation, the fast adaptive element is entrained by bundle creep, preventing rapid decay in the current. In contrast, probe excitation stiffens the HB, enabling submillisecond mechanical rise times and fast MET current adaptation. Differences in stereociliary morphology in our model, particularly the lack of transduction channels in the tallest row, suggested a role for the shortest row in enabling calcium-dependent slow adaptation. Indeed, predictions from a two-row isolated HB model (Tinevez et al., Biophys. J. 2007) optimized to generate creep and slow adaptation under fluid-jet excitation could not predict fast adaptation under stiff probe excitation, because of the lack of an additional mechanism for fast adaptation. Finally, from our model, the predicted activation curve width (90 nm), stiffness (11 mN/m), and the ratio of activation curve adaptive shift to applied adaptation step (0.52) aligned with experimental reports.

Conclusions: Our three-row model effectively predicts four characteristic time constants: the mechanical rise-time (the initial response to a step-input), mechanical creep, fast adaptation, and slow adaptation. The model provides mechanisms to describe the origin of each time constant and provides a physiologically grounded framework to include additional viscoelastic elements governing multi-protein interactions at both upper and lower tip link densities for embellishing the model in the future.

Unraveling the Calcium Dependence of Fast Adaptation in Outer-Hair-Cell Bundles

9:45 a.m. - 10:00 a.m.

Rayan Chatterjee¹, Daibhid O Maoileidigh^{*2}

¹*Stanford University School of Medicine*, ²*Stanford University*

Background: Normal hearing relies on outer-hair-cell hair bundles (OHBs). OHBs convert sound-induced forces into receptor currents, driving cochlear amplification. OHB receptor currents adapt in response to stimulation. Adaptation is thought to 1) maintain the ear's sensitivity to sound stimuli in the presence of step stimuli (for example, static pressure changes), 2) high-pass filter sound stimuli, 3) increase the ear's dynamic range, and 4) contribute to cochlear amplification. Despite its importance for hearing, the mechanisms causing OHB adaptation are unclear.

Some data show that the fastest components of adaptation depend on the extracellular and intracellular calcium concentrations but other data show that fast adaptation does not require calcium to enter the OHB during mechanotransduction. We propose that these apparently conflicting data can be reconciled if fast adaptation is a process that does not require calcium entry into the OHB and if the properties of fast adaptation change more slowly with the calcium concentration than the timescales of fast adaptation.

Methods: We develop a new mathematical model of the OHB demonstrating our proposal. The model includes two viscoelastic adaptation elements in series with the mechanotransduction channels through which the receptor current flows. The morphology of the model OHB is based on published experimental observations. The model fits 12 independent experimental data of different types, including the two timescales of fast adaptation, the weights of the fast adaptation components, and the extent of fast adaptation. The model quantitatively predicts an experimental observation not used to fit the model, the offset-induced displacement shift in the displacement-current curve, validating the model.

Results: The stiffness and damping coefficients of the adaptation elements set the timescales of fast adaptation, the weights of the fast adaptation components, and the extent of fast adaptation. We propose that decreasing the calcium concentration to physiological levels increases the stiffness of the adaptation elements. In agreement with additional experimental data on the effects of decreasing the calcium concentration, increasing the stiffness of the adaptation elements in the model increases the resting open probability of the mechanotransduction channels, increases the two timescales of fast adaptation, increases the weight of the longer fast-adaptation timescale, and increases the stiffness of the OHB. The model also predicts that increasing the stiffness of the adaptation elements, decreases the extent of fast adaptation, decreases the dynamic range of the OHB, increases the sensitivity of the OHB to stimulation, and changes the filtering caused by fast adaptation.

Conclusions: We find fast adaptation in OHBs is well-described by a model comprising viscoelastic elements that depend on the calcium concentration. This model accounts for 17 experimental observations and makes additional experimentally-testable predictions. The model resolves apparent conflicts in the literature about the calcium dependence of fast adaptation in OHBs.

Symposium 4: Inner Ear Glia and Their Roles in Hearing and Hearing Loss

8:00 a.m. - 10:00 a.m.

Meeting Room 209ABC, Floor 2

Inner Ear Glia and Their Roles in Hearing and Hearing Loss

Gabriel Corfas, *The University of Michigan, Kresge Hearing Research Institute*

Symposium Description: The central theme of the symposium is the exploration of inner ear glial cells, emphasizing their essential roles in maintaining cochlear function and how their dysregulation leads to hearing loss. The symposium will feature research on glial cell dysfunction in noise-induced hearing loss, the intersection of peripheral myelin disorders with hidden hearing loss, and genetic factors affecting glial cell function in auditory pathologies. Each presentation will highlight the mechanisms by which glial cells influence auditory nerve integrity, synaptic maintenance, and inflammatory responses that underlie both inherited and acquired hearing impairments.

This symposium is designed for a diverse audience of scientific and clinical professionals, including otolaryngologists, neuroscientists, audiologists, geneticists, and cell biologists. It is particularly relevant for researchers focused on auditory neuroscience, cellular and molecular biology, and translational medicine aiming to develop targeted therapies for hearing loss. Clinicians involved in diagnosing and treating hearing disorders will find value in the discussions of potential glial cell-related therapeutic targets. The symposium also welcomes students and early-career researchers seeking to deepen their understanding of how glial cell biology intersects with auditory function. Attendees will leave with insights into cutting-edge research and innovative strategies for addressing hearing loss through the study of inner ear glia.

Peripheral Myelin Disorders and the Mechanisms of Hidden Hearing Loss

Gabriel Corfas, *The University of Michigan, Kresge Hearing Research Institute*

Individual Abstract: Hidden hearing loss (HHL) is a recently identified auditory neuropathy believed to contribute to deficits in speech discrimination and intelligibility in people with normal audiological tests. In animal models, HHL presents as normal auditory thresholds but reduced sound-evoked potentials (ABR peak 1 amplitudes). Animal studies showed that age-related and noise-induced loss of synapses between the inner hair cells (IHCs) and spiral ganglion neurons (SGNs) correlate with HHL. Thus, IHC synaptopathy is often perceived as the only cause of HHL.

Since myelin impairments can also cause peripheral neuropathies, we tested whether myelin disorders cause HHL using three mouse models. First, Schwann cell ablation was induced by genetic means, causing a near-total loss of auditory nerve myelin within one week, followed by complete remyelination by four months. This demyelination does not alter auditory thresholds,

yet results in a permanent reduction of the amplitudes and increased latencies of the first peak of the auditory brainstem response (ABR). Importantly, transient demyelination does not cause IHC synaptopathy but produces a permanent disorganization of cochlear heminodes. Moreover, HHL caused by transient demyelination and noise are additive, indicating that HHL can be caused by at least two distinct mechanisms. Similar pathologies, i.e., HHL and disorganized heminodes, were found in a mouse model of Charcot-Marie-Tooth disease type 1A (CMT1A) and a mouse line that has peripheral nerve hypomyelination due to loss of Schwann cell ErbB receptor signaling.

Together, our results show that peripheral myelin alterations can cause HHL that is very similar to that seen with synaptopathy, except that it also affects ABR peak 1 latencies. It also suggests that the heminodes play a critical role in the generation of the auditory nerve compound action potential, possibly acting as the action potential initiation site, and that patients with peripheral myelin disorder might also suffer from HHL.

This work was supported in part by R01DC018500 (GC)

Auditory Phenotype in Neurofibromatosis Type 2 is Associated With Schwann Cell Dysfunction and Peripheral Myelinopathy

Judith Kempfle, *Massachusetts Eye and Ear, Harvard Medical School*

Individual Abstract: Background: Merlin, also known as neurofibromatosis type 2 (NF2), is a tumor suppressor gene. Its loss leads to the development of Schwann cell tumors, predominantly of the vestibular portion of the 8th cranial nerve. Schwannomas of the vestibular nerve are typically associated with varying degrees of sensorineural hearing loss (SNHL), but the etiology of this impairment remains to be elucidated. Leading current theories implicate the secretion of pro-inflammatory and potentially neurotoxic factors. In this study, we examined the auditory and vestibular nerves in a NF2 mouse model (Periostin-Cre; Nf2flox/flox) as well as human temporal bones to investigate the underlying cochlear NF2 phenotype with an emphasis on cochlear Schwann cells, and NF2 patients underwent hearing testing for subclinical hearing loss.

Methods: Periostin-Cre; Nf2flox/flox mice were compared to littermate controls with FVB/NJ background to characterize SNHL at 2 months and at later ages. For analysis of myelination, nodes of Ranvier and heminodes, cochlear whole mounts were stained for nodal (Gliomedin), paranodal (Caspr), and myelin (MBP) markers and imaged by confocal microscopy. Frozen cochlear sections were analyzed for glial and neuronal markers; Schwann cells and neurons were quantified per 100 μm area in Rosenthal's canal, Scarpa's ganglion, and the auditory nerve trunk on serial sections.

Human and murine temporal bones were stained for Schwann cell (Sox10) and macrophage markers, and ramification of macrophages was assessed to determine activation state.

Results: At 2 months of age, months prior to the onset of hearing loss and the development of proliferating Schwann cell tumorlets, Periostin-Cre; Nf2flox/flox mice have normal ABR and DPOAE thresholds[CG1], but reduced Wave I amplitudes and longer latencies in the auditory brain stem response than control mice. Analysis of heminodes and nodes of Ranvier was

consistent with myelinopathy in addition to beginning inner hair cell synaptopathy. At later ages, ABR demonstrated significant hearing loss in all NF2 mice compared to control mice.

Evaluation of mouse and human temporal bones revealed activation of macrophages in the disease ears, and patients with NF2 had abnormal speech-in-noise testing prior to overt hearing loss, concerning for early subclinical hearing loss.

Conclusions: Here we demonstrate that an NF2 mouse model and humans with NF2 display a cochlear phenotype that may be associated with dysregulation of myelination and a subsequent neuronal dysfunction that initially resembles synaptopathy and progresses to loss of spiral ganglion neurons. These findings may, in part, explain the SNHL in patients with vestibular schwannomas.

Glial Cell Malfunction in Noise-Induced Hearing Loss

Hainan Lang, *Medical University of South Carolina*

Individual Abstract: The auditory nerve (AN) is the primary conveyor of acoustic information from sensory hair cells to the brainstem. Aging, genetic defects, and cochlear insults from exposure to noise result in AN degeneration. The majority of AN fibers are myelinated by cochlear glial cells, allowing for fast conduction of electrical impulses along the AN. Our previous studies have demonstrated that noise exposure results in near-immediate myelin abnormalities. RNAseq analysis of a well-characterized mouse model of noise-induced hearing loss detected altered expression of numerous genes in the AN, including QKI, a central regulator of myelin formation, maintenance and repair. These interesting findings suggest an important contribution of myelinating glial cell dysfunction in noise-induced hearing loss. To further characterize the role of glial cells in noise-induced hearing loss, we examined transcriptional profiles of glial cells isolated from the AN of young adult Plp1/tdTomato mice after noise exposure. Comparison of glial cells isolated from the AN of noise-exposed mice to those of controls identified > 500 genes that were significantly changed. Top biological processes affected by noise exposure included inflammation and stress response. Further bioinformatic analysis identified versican, an extracellular matrix (ECM) molecule and direct QKI target, as a regulatory candidate of inflammation and myelin dysfunction in AN following noise exposure. Evaluation of versican expression during AN postnatal development found that isoforms V0 and V2 had unique expression patterns. We then examined young adult mice having deletion of versican isoforms V0 and V2 (V0/V2 KO mice), finding that they exhibited abnormal myelination, a delay in hearing onset, a mild loss of hearing sensitivity and expression changes in genes related to ECM function and inflammation. Additionally, auditory function recovery was reduced in adult V0/V2 KO mice following noise exposure. Together, these observations suggest a causal linkage between dysregulation of the QKI target versican, myelin abnormality, and hearing loss after noise exposure. Our study highlights the important role of AN glial cells in noise-induced hearing loss and other otologic disorders.

This work was supported (in part) by National Institutes of Health Grants R01 DC021436, P50 DC000422, T32 DC014435, P30 GM103342, P20 GM148302 and P20 GM103499, and a VA RR and D Merit Grant BX006478.

Genetic Polymorphisms in Human CX3CR1-Mediated Macrophage Dysregulation Are Associated With the Worsening of Hearing Loss and Cochlear Degeneration After Noise Trauma: A Study in a Humanized Mouse Model

Tejbeer Kaur, *Rutgers University, Robert Wood Johnson School of Medicine, Rutgers Brain Health Institute*

Individual Abstract: Sensorineural hearing loss (SNHL) is characterized by cochlear inflammation, macrophage activation, and degeneration of hair cells, synapses, and neurons. Macrophage-mediated inflammation in the damaged cochlea is regulated via CX3CR1-CX3CL1 signaling, where the fractalkine ligand CX3CL1 serves as a chemotactic and calming signal for macrophage activation. Furthermore, disrupted CX3CR1-CX3CL1 signaling in CX3CR1-KO and CX3CL1-KO mice leads to reduced macrophage numbers, exacerbated inflammation, and loss of hair cells, ribbon synapses, and neurons in the damaged cochlea. Notably, ~25% of the human population carries single-nucleotide polymorphisms (SNPs) in the CX3CR1 gene, CX3CR1I249/M280, which results in a receptor with lower binding affinity for CX3CL1, while most individuals carry the common wild-type CX3CR1V249/T280 allele. Although these polymorphisms are associated with various CNS neurodegenerative disorders, their impact on SNHL, cochlear degeneration, and the macrophage response remains largely unknown. Here, we used a humanized mouse model expressing human CX3CR1 SNPs in lieu of its murine counterpart to investigate the effects of I249/M280 polymorphisms on cochlear function and structure following noise trauma. Young CX3CR1 WT, CX3CR1 KO, and human CX3CR1I249/M280 mice were exposed to a noise level of 93 dB SPL for 2 hours at an octave band (8–16 kHz). We found that at 2 weeks postexposure, hearing thresholds were elevated and input–output function was impaired in hCX3CR1I249/M280 and CX3CR1 KO, whereas mice carrying WT alleles showed functional recovery. A significant synaptic loss (~30%) in hCX3CR1I249/M280 and CX3CR1 KO mice was observed relative to that in WT, which exhibited synaptic repair. hCX3CR1I249/M280 resulted in a ~17% loss of outer hair cells, which correlated with reduced otoacoustic emissions in the basal cochlear region. Noise led to increased macrophage numbers in the spiral ganglion and lateral wall of the WT; however, this response was attenuated in the CX3CR1 KO and hCX3CR1I249/M280 strains. Additionally, macrophages from CX3CR1 KO and hCX3CR1I249/M280 mice presented altered morphology, increased CD68 expression, and inflammation. Compared with those of mice carrying the CX3CR1 WT or KO allele, young hCX3CR1I249/M280 mice fostered under ambient noise presented early elevations in hearing thresholds at basal frequencies. Together, these findings reveal that human CX3CR1 variant-mediated macrophage dysregulation strongly correlates with worsening of hearing loss and cochlear degeneration after noise trauma. Our work proposes a novel immune-related genetic polymorphism that may aid in the identification of individuals with increased vulnerability to SNHL.

Podium 15: Vestibular: Basic Research and Clinical

8:00 a.m. - 10:00 a.m.

Ballroom B, Floor 3

Moderators: Phillippe Vincent and Alan Cheng

Cellular Heterogeneity and Spatial Organization of the Human Utricle

8:00 a.m. - 8:15 a.m.

Weisheng Liang^{*1}, Ryosuke Yamamoto², Emilia Luca², Alain Dabdoub³

¹*University of Toronto*, ²*Biological Sciences, Sunnybrook Research Institute*, ³*Biological Sciences, Sunnybrook Research Institute, Toronto, Canada, University of Toronto, Toronto, Canada*

Background: The utricle, a vestibular organ critical for balance, remains poorly defined at the molecular and cellular levels in humans, which limits progress toward the development of regenerative therapies for balance disorders. To address this gap, we employed single-nucleus multiomic sequencing and spatial transcriptomics to profile the fetal human utricle at early (gestational week 15) and late (weeks 18-19) stages, providing an integrated map of gene expression, regulatory dynamics, and cell organization in the human utricle.

Methods: We dissected sensory epithelia from gestational week (GW) 15 and 19 utricles (n = 2 each) and performed single-nucleus sequencing using the 10x Chromium Single Cell Multiome platform to simultaneously capture transcriptional (snRNA-seq) and chromatin accessibility (snATAC-seq) information for each cell. Dimension reduction and clustering were performed to characterize cell populations with distinct transcriptional and epigenomic signatures. Cell-cell communication was inferred with CellChat to identify ligand-receptor interactions. Based on bioinformatic analyses, we curated a customized 300-gene panel for imaging-based spatial transcriptomics and processed three utricles from each stage for high-resolution visualization of gene expression within intact tissue.

Results: Transcriptomic analysis identified distinct subpopulations of hair cells, supporting cells, and transitional epithelial cells, each defined by unique gene expression signatures and spatial organization. Neighbourhood analysis further delineated spatial niches within the sensory epithelium. A proliferative supporting cell population was observed at GW15 but declined at later stages. Leveraging accessible chromatin information, we uncovered epigenomic differences between the two stages and inferred regulatory networks between active transcription factors. In silico perturbation analysis further highlights candidate regulatory factors that may govern cell fate during utricle development.

Conclusions: Our integrative multiomic and spatial approach provides the first high-resolution view of the human fetal utricle, uncovering its cellular heterogeneity, gene regulatory dynamics, and spatial organization. These insights lay the groundwork for understanding human-specific mechanisms of sensory cell regeneration to inform strategies for treating balance disorders.

Transcriptional Differences Between the Human Otolith Organs and Ampullae Using Single Cell RNA-Seq

8:15 a.m. - 8:30 a.m.

Ruiqi Zhou^{*1}, Chisako Tanaka¹, Sushobhan Biswas¹, Tian Wang², Ankita Patro¹, Michael Canfarotta¹, Elizabeth Perkins¹, Kareem Tawfik¹, Aaron Moberly¹, Matthew O'Malley¹, Marc Bennett¹, David Haynes¹, Alan G. Cheng³, Taha A. Jan⁴

¹Vanderbilt University Medical Center, ²Stanford University, ³Stanford University School of Medicine, ⁴Epithelial Biology Center, Vanderbilt Center for Stem Cell Biology, Vanderbilt University Medical Center

Background: The human vestibular labyrinth is comprised of the utricle and saccule (otolith organs) and the three semicircular canals with their ampulla enlargements. These organs encode distinct modalities of head motion: the otolith organs detect linear acceleration and gravity, while the ampullae detect angular acceleration. Although all rely on mechanosensory hair cells (HCs), whether transcriptional programs differ among vestibular organs remains unknown in humans. In this study, we performed a computational analysis of single cell RNA-seq data comparing all 5 vestibular end-organs in humans.

Methods: IRB approval was obtained at Vanderbilt University Medical Center (VUMC, IRB#230391). We harvested inner ear vestibular epithelia from 14 patients that have undergone translabyrinthine resection of vestibular schwannoma tumors at VUMC. Samples were either directly processed for single cell RNA-seq or banked and processed using single nucleus isolation and subsequent RNA-seq. We further integrated this dataset with our previously published data of human utricles (Wang et al., 2024). Cell or nuclei capture was performed using droplet-based cell capture technique followed by library prep and Illumina sequencing. We applied deep learning and machine learning for analysis of datasets across patients to perform cell calling, quality control, batch correction, clustering, and cell-type annotation. We then focused on epithelial compartments, conducting organ-specific analyses to compare hair cells and supporting cells across the different organs. To assess organ level differences, utricle and saccule hair cells were integrated to define otolith hair cells and contrasted with ampulla hair cells, with parallel comparisons for supporting cells.

Results: We analyzed cells obtained from 18 ampullae, 5 utricles, and 4 saccules. Ten major cell types were annotated, including dark cells, hair cells, supporting cells, immune cells, macrophages, melanocytes, pericytes, roof cells, mesenchymal cells, and vascular cells. To mitigate the influence of ambient RNA, we applied CellBender for background correction. 8,117 cells passed all quality control metrics. The dataset shows a median of 1,945 genes detected per cell. Both scRNA-seq and single-nucleus RNA-seq were successfully integrated. In addition, we carried out comparative analyses between otolith organs and ampulla hair cells and supporting cells. Gene set enrichment analysis will be performed to test transcriptomic differences between the otolith and ampulla organs.

Conclusions: Our study provides a transcriptional atlas of human vestibular epithelia across the utricle, saccule, and ampulla. By integrating single-cell and single-nucleus RNA sequencing, we identified ten major cell types. Comparative analyses revealed organ-specific differences in hair cells and supporting cells of the otolith organs and ampulla. Our results provide new insight into vestibular organ specialization and cell type heterogeneity of the human inner ear.

Examining the Molecular Landscape of Vestibular Ganglion Neurons in an Adult Mammalian Model Using a Multiomic Approach

8:30 a.m. - 8:45 a.m.

Rahilla Tarfa*¹, Sarath Vijayakumar², MI ZHOU², David Raible¹, Litao Tao², Jennifer Stone³

¹University of Washington, ²Creighton University, ³University of Washington, Virginia Merrill Bloedel Hearing Research Center

Background: Some sensorineural vestibulopathies stem in part from damage to the primary sensory neurons—the vestibular ganglion neurons (VGNs). While we are aware of some functional and anatomical differences among mammalian VGNs, we know very little about their molecular properties or if there are molecularly distinct subtypes of VGNs. We are using single-nucleus multi-omics to define the transcriptomic and chromatin accessibility profiles of these little-studied neurons and in situ hybridization and immunolabeling to relate these profiles to VGN anatomy in adult mice.

Methods: Bilateral vestibular ganglia were isolated from seven C57BL/6 mice (2 months old) and processed using 10x Genomics Single-Cell Multiome (ATAC + Gene Expression). Data were analyzed in R using Seurat v5.0 for scRNA-seq and Signac v1.0 for scATAC-seq (mm10). Initial data were examined and filtered using quality control metrics. We then constructed a gene-activity matrix for ATAC-seq data and performed cross-modal label transfer to the RNAseq clusters. Cell types were annotated manually from canonical markers and further refined by cross-modality transfer.

Results: ATAC-seq revealed distinct chromatin accessibility clusters corresponding to neurons, myelinating and non-myelinating Schwann cells, satellite glia, immune cells, and endothelial cells. Labels transferred between modalities showed barcode concordance for VGNs (ATAC-seq→RNA-seq 52%, and RNA-seq→ATAC-seq 57% of VGNs) with a median prediction score of 0.963 (IQR 0.905–0.990). The two neuron-enriched clusters from the RNA-seq data were then isolated, re-embedded, and re-clustered, yielding three putative VGN subclusters. Candidate markers were defined by joint criteria ($\log_2FC > 1.5$ and average expression > 1.0), as subtype-specific genes for validation.

Conclusions: By comparing snRNAseq and ATACseq data from the same cells in mouse vestibular ganglia, we defined three distinct VGN subtypes. We are employing in situ hybridization to validate genes whose expression is predicted to distinguish VGNs from glia, and to validate differential gene expression in the three putative VGN subtypes. We are also working to correlate differentially expressed genes in each neural subtype with its anatomic properties, including size, morphology, and position in the ganglion.

The Cellular and Molecular Architecture of the Mammalian Vestibular System

8:45 a.m. - 9:00 a.m.

Meghna Kolluri^{*1}, Paula Fontanet¹, Prach Techameena¹, Csaba Adori¹, Laurence Picton¹, Charles Petitpre¹, Shih-Hsin Chang¹, Haohao Wu¹, Beatriz Del Blanco², Angel Barco², Saida Hadjab¹, Francois Lallemand¹

¹Karolinska Institutet, ²Universidad Miguel Hernández

Background: Our sense of balance is governed by the vestibular system located in the inner ear. It consists of three semi-circular canals (SCCs) and two otolith organs i.e. the saccule and the utricle. In each organ, the sensory epithelium harbors the mechanosensory vestibular hair cells (VHCs) that detect head movements. The mechanical stimuli, generated upon head movement,

are converted to electrical signals that are relayed to higher processing centers in the hindbrain via vestibular afferent neurons (VANs). Although the diversity of VHCs and VANs, with respect to their morphology and physiology, has been described for decades, their molecular classification, and therefore functional organization, has remained unclear.

Methods: We combined single-cell RNA sequencing, immunohistochemistry, viral tracing, 3D light-sheet imaging, and in vitro electrophysiology to investigate vestibular neurons and hair cells in mice. This integrated approach revealed the diversity of cell types, defined the morphology, spatial organization, connectivity, and developmental origins of the newly identified VAN and VHC subtypes, and characterized their functional properties.

Results: We identify 11 neuronal and 9 hair cell types in the vestibular system. Distinct innervation and spiking patterns among VAN subtypes highlight the complexity of vestibular input and processing, suggesting specialized contributions to balance and gaze stabilization. Novel VHC subtypes display distinct spatial distributions across and between the sensory epithelia, adding another layer to vestibular perception. At birth, six neuronal subtypes are present, representing major groups. During the first postnatal week, as motor behaviors emerge, these groups remain highly fluid between one another and undergo complex, non-linear developmental dynamics before stabilizing and giving rise to additional subtypes by the second week.

Conclusions: Our research aims to delineate the cell type-specific network architecture of VHCs and VANs and elucidate the multiple pathways that produce an integrated vestibular motor output and provide a basis for pharmacological research and potential drug targets in vestibular disease conditions.

Group 1 RFX Transcription Factors are Essential for Kinocilia Formation and Vestibular Function

9:00 a.m. - 9:15 a.m.

Lesly Umanzor^{*1}, Kathleen Gwilliam¹, Beatrice Milon¹, Han Dewan¹, Mai Eshel², Shaked Ashur², Mark McMurray³, Yuliya Sokolova¹, Inna Belyantseva¹, Sherri Jones⁴, Ran Elkon², Ronna Hertzano¹

¹*Section on Omics and Translational Science of Hearing, Neurotology Branch, National Institute on Deafness and Other Communication Disorders, National Institutes of Health, Bethesda, MD 20892, USA*, ²*Tel Aviv University School of Medicine, Tel Aviv University, Tel Aviv, Israel*, ³*University of Maryland School of Medicine, Baltimore, MD, USA*, ⁴*University of Nebraska Lincoln, Lincoln, Nebraska, USA*

Background: Developing therapeutics for hearing and balance disorders requires understanding transcriptional regulatory networks governing hair cell (HC) development. We previously identified Group 1 regulatory factor X (RFX) transcription factors (TF) motifs, RFX1, RFX2, and RFX3, in the promoters of cochlear and vestibular HC-expressed genes, suggesting roles in HC development and maintenance. Conditional deletion of Rfx1 and Rfx3 (Rfx1/3; Gfi1-Cre) caused profound hearing loss, rapid outer hair cell loss at hearing onset, and a mild late-onset vestibular phenotype. Given their homology, shared ciliogenesis roles, and cochlear and vestibular HCs expression of all group 1 RFX TFs, we hypothesized that RFX2 compensates for

the loss of RFX1/3. Here, we examined the role of group 1 RFX TFs in kinocilia development in the vestibular system and their downstream signaling cascade.

Methods: To assess Rfx2 compensation in the vestibular system, we generated a triple conditional knockout (cKO) mouse of group 1 RFX TFs. Vestibular function was evaluated using vestibular sensory evoked potentials (VsEPs), and tissues from early postnatal ages to 6 months were analyzed by immunohistochemistry and scanning electron microscopy. Kinocilia length at P(5) was quantified using Imaris filament tracer. To define downstream transcriptional cascades of the group 1 RFX TFs, we performed single-cell RNA-sequencing (scRNA-seq) on utricular and saccular sensory epithelia from P5 Rfx1/2/3 cKO and control mice using the 10x Genomics platform, with subsequent analysis with Seurat v4.

Results: Loss of group 1 RFX caused elevated VsEP thresholds detected at 1 month of age, which worsened at 3 and 6 months. Histologically, at P5, utricular kinocilia in the Rfx1/2/3 cKO mice were significantly shorter in both striolar (Mann-Whitney U test, $H(df) = -4.75$, $p < 0.0001$) and extrastriolar regions ($H(df) = -4.90$, $p = 0.0001$). scRNA-seq of P5 Rfx1/2/3 cKO mice further revealed downregulation of genes involved in cilium organization and movement. Gene Ontology enrichment analysis highlights dysregulation of ciliary assembly, trafficking, and lipid metabolism. Promoters of the downregulated genes were enriched for the RFX binding signature, while no significant enrichment was identified in upregulated genes, consistent with the role of RFX as a transcriptional activator.

Conclusions: Our findings indicate that RFX2 compensates for RFX1/3 loss in the vestibular system and that group 1 RFX TFs are essential for vestibular function and kinocilia development. Elevated VsEP thresholds in mutants suggest a possible role for vestibular kinocilia in HC function; however, the loss of function of the cKO HCs could also result from additional pathways regulated by RFX1/2/3. Furthermore, we identified components of the group 1 RFX signaling pathway in vestibular HCs, including candidate genes involved in kinocilia maintenance and vestibular function. Together, these results provide mechanistic insight into hair cell ciliogenesis and highlight potential pathways underlying the vulnerability of hair cells to sensorineural hearing loss and vestibular dysfunction.

3D-Ultrastructural Organization of the Macula Utricle Supporting Cell Cytoplasmic Granules

9:15 a.m. - 9:30 a.m.

Ivan Lopez^{*1}, Jinlin Pan¹, Gail Ishiyama², Akira Ishiyama², Larry Hoffman¹

¹David Geffen School of Medicine at University of California, Los Angeles, ²UCLA

Background: The mammalian vestibular sensory epithelium contains hair cells and supporting cells (SCs). SCs play several essential roles: (a) otoconia and cupula formation, (b) acting as hair cell precursors, and (c) structural support. Previous scanning electron microscopy studies had revealed secretory granules in the cytoplasm of vestibular SCs (Takumida and Anniko, *Acta Otol* 114:2,150-155,1994). These granules contain high calcium contents and are thought to be extruded from the apical surface of the maculae to form the otoconial crystals. In this study we investigate the 3D subcellular organization of the secretory granules in mouse utricular SCs and identify putative proteins within these granules in the human utricle SCs.

Methods: Forty nanometer thick plastic serial sections (n=400) of the mouse utricle (C57BL/6 mice) vestibular sensory epithelium were prepared using ATUM-SEM as described by Terasaki et al. 2012, Kastouri et al. 2014). Digital images were acquired using a field emission scanning electron microscope. 3D reconstructions of the SCs were generated using VAST Lite software for the segmentation of the granules within the supporting cell cytoplasm and AMIRA software was used to generate 3D images. Mitochondria adjacent to the granules were also segmented for 3D reconstruction. Macula utricles obtained from autopsy or ablative surgery were fixed and 20-micron thick cryosections were used for immunofluorescence (IF) staining. Supporting cells were identified using monoclonal antibodies against GFAP, and polyclonal antibodies against otopetrin-2 (Lopez et al 2019, *Ann Otol Rhinol Laryngol*, 128:96-102). Images were acquired using a Leica SP8 confocal light sheet microscope. Human macula utricles were also processed for transmission electron microscopy.

Results: In the mouse utricle SCs granules were present from the apical to the nuclear region, the granules were almost absent at the basal portion of the cytoplasm. Granules were spherical in shape, not interconnected, and segregated to the apical portion of the cytoplasm. Interestingly mitochondria were segregated to the periphery of the SCs cytoplasm. TEM images of the human macula utricle revealed the presence of the SCs granules at the apical and nuclear region. Punctate otopetrin-2-IF was present in the SCs cytoplasm, GFAP allowed the identification of SCs and did not colocalize with otopetrin-2.

Conclusions: This study reveals, for the first time, the 3D ultrastructural organization of secretory granules in mouse utricular SCs, and their presence in the human macula utricle. Otopetrin-2 may be among the proteins present in the SCs granules. Insights into SC organization may inform future strategies for hair cell regeneration and cell survival after ototoxic damage or aging.

Apoe-High Schwann Cell Subpopulations Promote Cystic Transformation in Vestibular Schwannoma: A Single-Cell Transcriptomic Perspective

9:30 a.m. - 9:45 a.m.

Jiaqi Pang¹, Junbo Wang^{*2}, Yiqing Zheng³, Gen Li³, Maojin Liang³

¹*Sun Yat-sen University, Sun Yat-sen Memorial Hospital*, ²*SYSU 2nd Memorial Hospital*, ³*Sun Yat-sen Memorial Hospital*

Background: Sporadic vestibular schwannoma (VS), the most common cerebellopontine angle tumor, can compress adjacent cranial nerves and brainstem structures, leading to progressive hearing loss, facial nerve dysfunction, and life-threatening complications. Magnetic resonance imaging (MRI) allows classification into cystic (CVS) and solid (SVS) subtypes based on distinct radiological features: CVS exhibits cystic components with hyperintensity on T2-weighted imaging and heterogeneous enhancement patterns, whereas SVS presents as homogeneous solid masses. Clinically, CVS demonstrates accelerated growth rates (2-6 mm/year), higher KOOS grading, and earlier onset of brainstem compression compared to SVS. However, the tumor microenvironment (TME) characteristics underlying this aggressive phenotype remain poorly characterized.

Methods: Through single-cell RNA sequencing of human CVS, SVS, and trigeminal ganglion tissues, we constructed a cellular atlas of cranial nerve pathologies.

Results: Comparative analysis revealed CVS-specific TME features: (1) Schwann cell heterogeneity dominated by Schwann_N APOE subclusters; (2) Significant enrichment of myeloid cells (predominantly Mac CCL3 and Mac SDS subtypes) and T/NK cell populations. Computational analysis via CICA identified reciprocal crosstalk between Mac CCL3/SDS and Schwann_N APOE through TNF and MIF signaling pathways.

Conclusions: We propose that TNF-MIF axis-mediated neuroimmune interactions drive cystic degeneration via inducing schwann cell necrosis, which may explain the voluminous cystic components characteristic of CVS. Importantly, myeloid cell abundance positively correlated with tumor size ($p < 0.01$) and KOOS grade ($r=0.72$), suggesting their role as biomarkers for disease progression.

Use of Artificial Intelligence Hearing Aids for Gait Analysis and Fall Risk

9:45 a.m. - 10:00 a.m.

Matthew Fitzgerald¹, Jwala Rejimon*¹, Majd Srour², Varsha M Athreya³, Achintya Bhowmik⁴, David Fabry², Robert Jackler¹, Kristen Steenerson¹

¹Stanford University School of Medicine, ²Starkey Hearing Technologies, ³Stanford University, ⁴Starkey Hearing Technologies; Stanford University

Background: Falls are a leading cause of injury in adults, making fall-risk identification critical. The CDC's STEADI protocol uses validated assessments to predict fall risk. However, limited resources, extended appointment times, and required training hinder STEADI's clinical implementation. Previously, we evaluated AI hearing aids with inertial sensors (IMU-HAs) for remote fall-risk assessment via smartphone app. These devices incorporated self-administered fall-risk assessments, demonstrating strong agreement between IMU-HA fall-risk scores and clinicians' scores. The next step towards scalable fall prevention is to enable hearing aids to classify fall-risk through passive monitoring of daily movements. In this study, we aimed to validate the IMU-HA gait analysis feature against gold-standard gait sensing devices and identify which mobility features that are easily identified by the hearing aids are most predictive of fall risk.

Methods: Participants ($n = 30$) were recruited to complete a battery of validated mobility tasks while wearing both IMU-HAs and Mobility Lab's Clario sensors positioned at the lumbar region and both feet. Tasks included a gait speed test, timed transitions between sitting/laying/standing, static balance exercises, timed chair stands, and the Timed-Up-And-Go (TUG). The Functional Gait Assessment was also administered by a trained clinician to provide a ground-truth classification of the participants' gait and fall risk (grouped into high- or low-fall risk groups). IMU-HA measurements were first compared with the Clario sensor measurements to establish validity. Then performance on the mobility tasks in both groups were compared against FGA classifications to identify predictive features of fall-risk.

Results: Preliminary analyses demonstrate strong agreement between IMU-HAs and Mobility Lab sensors for step time and cadence, consistent with findings from our pilot study ($r = 0.92$ for right step time; $r = 0.94$ for left step time; $r = 0.98$ for cadence). Agreement was weaker for

variables such as double support time and stance asymmetry. Work is ongoing to examine predictors of fall risk based on FGA classifications, with early trends suggesting that TUG performance and transitional movement times are particularly discriminative.

Conclusions: IMU-equipped hearing aids show promising validity for measuring key gait parameters associated with fall risk. Early evidence suggests that these devices can capture clinically meaningful mobility features, providing a path toward passive, unobtrusive fall-risk monitoring in daily life. Continued analysis will clarify which variables best classify individuals as high or low risk, supporting the development of scalable, hearing aid-based fall prevention strategies.

Podium 16: Inner Ear Physiology and Pathology: Mechanisms of Synaptic Transmission, Energetics, Cochlear Amplification and Hearing Loss

10:30 a.m. - 12:30 p.m.

Meeting Room 209ABC, Floor 2

Moderators: and **Kazuaki Homma**

CHD7 Promotes Recycling of Synaptic Vesicles in Ribbon Synapses of Inner Hair Cell in the Mouse Cochlea

10:30 a.m. - 12:30 p.m.

Yige Li^{*1}, Shihang Mao¹, Fang Wang¹, Mengfan Xu¹, Shan Sun¹, Geng-Lin Li¹

¹*Eye and ENT Hospital, Fudan University*

Background: Mutations in the gene encoding chromodomain helicase DNA binding protein 7 (CHD7) cause the CHARGE syndrome, an autosomal dominant inherited disease characterized by multiple developmental deficits, including sensorineural hearing loss (SNHL). However, functional mechanisms of CHD7 in SNHL is poorly understood.

Methods: We crossed the Chd7f/+ mice with the Dppa3Cre/+ mice in the C57BL/6J background and used the Chd7f/+ mice and the Dppa3Cre/+; Chd7f/+ mice as the control (CTRL) and experimental group (HET), respectively. Mice of 4 weeks old and both sexes were used. To assess structural and functional changes, we recorded auditory brainstem responses (ABRs), performed immunolabeling in whole-mount cochleae, conducted patch-clamp recording in inner hair cells (IHCs) in whole-mount cochleae and in bushy cells in the cochlear nucleus in brainstem slices.

Results: Firstly, we found that in HET mice ABR thresholds were significantly elevated (17 ~ 40 dB) for both clicks and pure tones of all frequencies, amplitudes of both Wave I and II reduced, and latencies increased for Wave I but not Wave II, resulting in shortened interval between Wave I and II. Furthermore, the count of neurofilaments was significantly reduced in the apical turn (11.4 vs 7.76), with no change observed in count or morphology of hair cells in all the three turns. Secondly, we found significant reduction in both the peak amplitude of calcium current (ICa, 220 vs 190 pA) and the exocytosis in IHCs (assessed as membrane capacitance increase, ΔCm, 49.2 vs 25.4 fF, for depolarization of 200 ms). Furthermore, the ratio of

$\Delta C_m/QCa$ was also significantly reduced (2.15 vs 1.23 fF/pC), suggesting that the Ca^{2+} efficiency in triggering exocytosis was decreased. Thirdly, we applied curve fitting to examine dynamics of exocytosis, and we found that while the readily releasable pool (RRP) of synaptic vesicles (SVs) was unchanged (385 vs 311 SVs), the sustained release rate (SRR) of SVs was significantly reduced (4,670 vs 2,186 SVs/s), suggesting that recycling of SVs was decreased. To examine how exocytosis is impacted in response to physiologically more relevant simulation, we applied 50 short depolarizations of 5 ms, separated by 10 ms intervals, and we found that exocytosis was significantly decreased (109 vs 73.9 fF). Lastly, we examined synaptic transmission at the endbulb of Held synapse, the first central synapse in the auditory pathways, and we found no significant change in the quantal size, synaptic strength or excitability of postsynaptic bushy cells.

Conclusions: In summary, we found that in the mouse cochlea, haploinsufficiency of CHD7 caused significant reduction in the SRR of SVs without changing the RRP of SVs. We conclude that CHD7 promotes recycling of SVs in ribbon synapses of IHC in the mouse cochlea.

Giant Capacitance Changes Reveal Coordinated Multivesicular Release in Inner Hair Cells

10:45 a.m. - 11:00 a.m.

Wonchul Shin*¹, Catherine Weisz¹

¹*National Institutes of Health*

Background: Inner hair cell (IHC) ribbon synapses sustain high rates of neurotransmission with extraordinary temporal precision. Postsynaptic recordings from type I spiral ganglion neurons have revealed unusually large and heterogeneous excitatory postsynaptic currents (EPSCs), showing multivesicular release (MVR) at these synapses. Yet, the extent and temporal resolution of MVR at IHC synapses remain incompletely understood.

Methods: Whole-cell patch-clamp recordings were performed in voltage clamp mode using voltage step -80 mV to -10 mV with 100 ms trains of depolarization. Membrane capacitance (C_m) and Ca^{2+} current (I_{Ca}) were simultaneously measured to observe exocytosis activity in mouse IHCs at postnatal days of 6-9 (p6-p9).

Results: We observed striking giant C_m increases during 100 ms trains of depolarization. In ~40% of cells, individual 100 ms steps in the trains triggered giant increases of ~100–400 fF during some of the stimulations, corresponding to fusion of 2,500–10,000 vesicles—far exceeding the amplitudes usually seen with isolated single 100 ms stimulation. These giant C_m increases occurred both during 100-ms trains and, rarely, during the very first step of trains or a single depolarization when Ca^{2+} influx was particularly large. Such kinetics may imply that these responses require rapid and massive recruitment of vesicles within the time window of 100 ms stimulation. The largest events (~400 fF) show that IHCs can release ~10,000 vesicles within 100 ms, equivalent to ~500 vesicles per ribbon assuming ~20 active ribbons per cell. This indicates approximately one vesicle fusion every 0.2 ms per ribbon, supporting the view that IHC synapses are capable of highly coordinated MVR at sub-millisecond resolution.

Conclusions: IHC ribbon synapses may operate in at least two MVR modes: (1) conventional MVR under weak or moderate stimulation, and (2) coordinated, large-scale MVR that occurs

under strong or sustained stimulation. This latter mode may involve highly synchronized exocytosis of large vesicle ensembles, potentially by compound fusion.

These findings suggest that the extraordinary output of IHC synapses arises not only from conventional MVR release but also from a large-scale multivesicular release. This mechanism may serve as an alternative mode of synaptic transmission, ensuring robust encoding of sudden or extremely strong acoustic signals while preserving temporal fidelity. As these experiments were conducted in IHCs from P6-P9 mice, it will be important to determine whether such giant Cm events represent a feature of the developmental stage or are also preserved after the onset of hearing in mature animals. Further validation of these mechanisms will require super-resolution imaging to directly visualize vesicle fusion at ribbon active zones.

Temporal Control of Ribeye Deletion Unmasks the Essential Role of the Synaptic Ribbon in Hearing

11:00 a.m. - 11:15 a.m.

Najate Benamer*¹, Wei Li¹, Pierre Platel¹, Loick Thibonnier¹, Jeremie Barral¹, Saaid Safieddine¹

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Background: The inner hair cell (IHC) ribbon synapse is a specialized structure that enables rapid, and sustained neurotransmitter release, essential for high-fidelity auditory signaling. Its hallmark is the synaptic ribbon, an electron-dense structure composed of RIBEYE, which tethers synaptic vesicles near voltage-gated Cav1.3 calcium channels to ensure a continuous supply for glutamate release. While ribbon synapse dysfunction is implicated in auditory synaptopathies, recent findings show that IHCs from mice with constitutive RIBEYE deletion can retain normal transmitter release and hearing (Jean et al, Becker et al; 2018), suggesting either limited ribbon function or developmental compensatory mechanisms. To address this, we generated a conditional knockout (cKO) mouse model enabling temporal control of RIBEYE deletion in sensory hair cells.

Methods: Synapse organization was analyzed using confocal imaging and ultrastructure analyses by electron microscopy. Hearing function was assessed by auditory brainstem responses (ABR), while IHC biophysical properties were characterized using patch-clamp and membrane capacitance measurements. Central auditory processing was evaluated using in-vivo high-density electrophysiological recordings (Neuropixels) in two central relays of the auditory pathway, the cochlear nucleus (CN) and inferior colliculus (IC). Auditory perception was assessed through a Go-NoGo auditory frequency discrimination task.

Results: Consistent with previous studies using constitutive RIBEYE Knockout mice, RIBEYE deletion at postnatal day 2 (P2) did not affect ABR thresholds but reduced wave I amplitudes, despite preserved IHC exocytosis. In contrast, RIBEYE deletion after hearing onset (P15) led to severe to profound deafness and a drastic reduction in IHCs synaptic exocytosis.

Neuropixels recordings revealed that, following RIBEYE loss at either developmental stage, both the CN and IC neurons exhibited delayed onset spike latencies and reduced firing rates. Although synchronization indices at low frequencies remained similar to controls, both early and

late RIBEYE cKO groups showed fewer phase-locked units. These alterations were associated with impaired neural entrainment and decreased temporal synchrony in both structures, particularly in mice with RIBEYE post-hearing onset deletion. At the behavior level, mice with neonatal RIBEYE deletion retained frequency discrimination abilities, whereas mice with ribeye disruption after hearing onset were unable to perform the task.

Conclusions: Our findings demonstrate that the timing of RIBEYE deletion critically determines the functional outcome of IHC ribbon synapses disruption. Neonatal deletion engages compensatory mechanisms that preserved IHC synaptic exocytosis and perceptual acuity, despite reduced ABR wave I amplitudes and altered spike timing. In contrast, deletion after hearing onset causes severe to profound deafness, collapse of IHC exocytosis and impaired neural entrainment leading to loss of auditory perception.

This highlights a developmental window during which the auditory system can compensate for ribbon loss, underscoring the plasticity of immature cochlear circuits. Beyond this window, however, the mature auditory system is critically dependent on RIBEYE-mediated synaptic architecture to sustain temporal fidelity, neural synchrony, and perceptual acuity.

Measuring Respiratory Capacity and Energetic Demands in Tissues of the Murine Cochlea Using High Resolution Respirometry and Fluorometry

11:15 a.m. - 11:30 a.m.

Enrique Rodriguez¹, Claire Scott², Zoe Mann², James O'Sullivan^{*2}

¹University College London, ²King's College London

Background: Mitochondrial dysfunction in the cochlea is a hallmark of both ototoxicity and age related hearing loss. However, we do not yet fully understand how these challenges might 1) alter the metabolic demands of normal cochlear tissues, and 2) disrupt the capacity of mitochondria to support these demands through oxidative phosphorylation. Confocal microscopy and enzymatic assays have been instructive in characterising the progression of mitochondrial dysfunction, but new methodological approaches are needed to understand whether specific metabolic pathways are directly affected by insults. Here, we investigated the feasibility of high resolution respirometry to measure respiratory substrate usage and activities of electron transport chain (ETC) complexes in cochlear tissues using the Oroboros-O2k platform.

Methods: Cochleae were obtained between postnatal days 3-5 (P3-5) from outbred CD1 mice. Two types of cochlear preparation were analysed: 1) whole tissue homogenates of the cochlear duct (including the sensory epithelium and stria vascularis) as well as the spiral ganglion, and 2) isolates of the crude mitochondrial fraction refined from homogenates using centrifugation. Samples were assessed using an Oroboros-O2k respirometer equipped with a 465 nm excitation LED and Fluo Sensor Blue, allowing simultaneous measurement of O2 flux via amperometric O2 detector and rates of ATP production via fluorescence of the dye Magnesium Green. During each experiment, preparations were sequentially exposed to respiratory substrates, the mitochondrial uncoupler CCCP, and inhibitors of ETC complexes. Data were normalised to protein content of mitochondrial preparations.

Results: All crude mitochondrial fractions exhibited prominent complex II (CII)-linked respiration, with oxygen consumption being mostly driven by succinate. This CII-linked

respiration was especially pronounced in the spiral ganglion, whilst cochlear duct preparations exhibited a larger component of CI-linked oxygen consumption. Reflecting this difference, pyruvate and malate drove ATP synthesis only in the cochlear duct, whilst the spiral ganglion relied solely on succinate. Whole-tissue homogenates displayed a marked shift in oxygen consumption and ATP dynamics compared with isolates of the crude mitochondrial fraction. Oxygen use was almost entirely driven by CII, implying that cytosolic lactate dehydrogenase A is able to rapidly convert excess pyruvate to lactate, providing further evidence of its role as a key regulator of cochlear metabolic homeostasis. Homogenates also continuously consumed ATP, and quantifying this rate after the addition of the F1Fo ATP synthase inhibitor oligomycin offered a direct readout of cochlear energy demand.

Conclusions: Here we establish high-resolution respirometry as a viable approach for probing cochlear bioenergetics. Different sample preparation techniques revealed distinct patterns of tissue-specific ETC complex activity as well as ATP demand. Future work will employ the technique to analyse the effects of ototoxins on respiratory complexes and energy consumption of inner ear tissues, potentially identifying targets for therapeutic intervention.

Audiogenic Seizures in Prestin Mouse Models

11:30 a.m. - 11:45 a.m.

Kazuaki Homma^{*1}, Satoe Takahashi², Yingjie Zhou¹, Frédéric Dépreux², Donna Whitlon², Mary Ann Cheatham¹

¹*Northwestern University*, ²*Northwestern University Feinberg School of Medicine*

Background: Prestin (SLC26A5) confers sound-elicited somatic electromotility on cochlear outer hair cells (OHCs) and is essential for the exquisite sensitivity and frequency selectivity of mammalian hearing. Lack of prestin results in ~50 dB hearing threshold elevations across frequency, supporting the causal association of the prestin-coding gene, SLC26A5, with hereditary hearing loss, DFNB61. Previously, we generated A100T- and P119S-prestin knock-in (KI) mouse models and showed that homozygotes of both lines exhibited not only impaired hearing but also susceptibility to audiogenic seizures. In this study, we extended our characterization to other DFNB61 mouse models to better understand their susceptibility to audiogenic seizures.

Methods: Mice were placed in a plastic testing cage (28Lx18Wx13H in cm) with a metal mesh lid housed in a sound-attenuating chamber (Acoustic Systems, ETS-Lingren). Before noise exposure, mice were allowed to acclimate to the testing cage for at least 15 seconds. A 950NeoPB 8Ω compression driver (Radian Audio Engineering) placed 15 cm above the testing cage was used to deliver 110 dB SPL white noise for up to 20 seconds per experiment. To produce the white noise, the electrical output from a Hewlett-Packard 33120A waveform generator was amplified by a Hafler P1500 Trans Nova power amplifier. The sound level (110 dB SPL) was flat between 0.5 kHz and 20 kHz with approximately -30 dB SPL per octave reductions beyond this frequency band (< 0.5 kHz and > 20 kHz) due to the mechanical characteristics of the compression driver. Sound pressure level was monitored using a 1/8-inch Brüel and Kjær microphone or the analog RadioShack sound meter 33-4050. Audiogenic seizure phenotype was qualitatively categorized as follows: 0) no response; 1) wild-running; 2) wild-running with jumping; 3) tonic-clonic phase. For noise-sensitive mice, stimulation was stopped

(typically < 5 seconds) at the onset of wild-running with jumping to prevent death from the seizures.

Results: Prestin null, A100T-KI, P119S-KI, and R130S-KI homozygotes all exhibited sensitivity to audiogenic seizures at three to four weeks of age, with fewer mice exhibiting seizure phenotypes at older ages. Some heterozygotes exhibited hints of irritation at weaning but were largely unresponsive. The seizure phenotype depends on synaptic output from the cochlea, as double knockout mice lacking prestin and VGLUT3 did not exhibit audiogenic seizures at any age tested.

Conclusions: We have uncovered the previously underappreciated phenotype of audiogenic seizures in prestin-deficient mice. As prestin variants have been implicated in autism spectrum disorders that are often presented with overt sensitivity to sound, our DFNB61 mice can serve as exciting new models to elucidate mechanisms underlying acoustic hypersensitivity and its possible amelioration in human patients.

This work is supported by NIDCD (DC017482 to KH), ASD[HA] (W81XWH-20-1-0484 to DW), and the Knowles Hearing Center.

Impact of Disrupted Otoferlin Ca²⁺ Binding on Inner Hair Cell Synaptic Transmission

11:45 a.m. - 12:00 p.m.

Abigail Trebilcock^{*1}, Han Chen², Fritz Benseler³, Nils Brose³, Tobias Moser¹

¹*Institute for Auditory Neuroscience, University Medical Center Goettingen*, ²*Institute for Auditory Neuroscience and InnerEarLab, University Medical Center Göttingen, Göttingen, Germany*, ³*Max Planck Institute for Multidisciplinary Sciences, Göttingen, Germany*

Background: The specialized ribbon synapse between inner hair cells (IHCs) and spiral ganglion neurons (SGNs) possesses a unique synaptic machinery that supports indefatigable sound encoding with high temporal precision. IHCs contain otoferlin, a Ca²⁺-sensitive multi-C2-domain protein that is a common target of human deafness mutations leading to auditory synaptopathy. Although human deafness mutations and mouse models show that otoferlin is essential for IHC exocytosis, the role of otoferlin as the IHC Ca²⁺ sensor remained to be tested and the contribution of the C2D domain was unknown. In this study we interrogated the role of the C2D domain by investigating the effect of disrupted C2D Ca²⁺ binding on synaptic transmission.

Methods: Using site-directed mutagenesis, we generated three Otof mutant mouse lines, OtofD1060A, OtofD990A, OtofD990-996A in which aspartate residues thought to coordinate Ca²⁺ were replaced with alanine to reduce the Ca²⁺-binding affinity in the C2D domain. Since the vast majority of type I SGNs receive input from a single IHC, in vivo physiological recordings of SGN firing activity were used to interrogate IHC-SGN synaptic transmission. This included evaluation of SGN activity at the population level, with auditory brainstem response recordings (ABRs) and at the single cell level with juxta cellular recordings of single SGN fibers.

Results: ABR thresholds were slightly to moderately elevated in the three Otof mutant strains, to a degree consistent with the structure-predicted extent of impairment of C2D Ca²⁺ binding. Wave I amplitude, but not wave IV amplitude, was substantially diminished indicating reduced SGN synchronicity. At the single SGN level, spontaneous firing tended to be lower in OtofD990A and OtofD990-996A mutants. Sound-evoked responses of OtofD990A and OtofD990-996A mutants to 50 ms tone bursts showed impaired onset coding. This included lower onset firing rate and longer first spike latency with increased variance. Comparatively, the adapted firing rate was relatively intact. Due to the preferential impairment in onset response, not adapted response, we conclude that IHC vesicle replenishment is relatively intact and that reduced release probability, not RRP size, drives the diminished onset firing. Single exponential fitting of spike rate adaptation showed a slower time constant, further supporting a reduction in release probability.

Conclusions: Impaired sound onset coding indicates a reduced release probability at IHC-SGN synapses in Otof mutants with altered C2D Ca²⁺ binding. This supports the Ca²⁺-sensor hypothesis for otoferlin and illustrates the importance of the C2D domain in this function. Next, we will fit the SGN responses with a biophysical model of SGN firing activity to predict RRP size, release probability, and vesicle replenishment rates. In the context of recent developments in otoferlin gene therapy, better understanding precise functions of otoferlin and the roles of each C2 domain provides a foundation for potential personalized treatment for specific otoferlin mutations.

Mechanistic Insights Into Sensorineural Hearing Loss in Meniere's Disease From a Murine Model of X-Linked Hypophosphatemia

12:00 p.m. - 12:15 p.m.

Ashwin Jhawer^{*1}, Arpan Bose², Frank Yeh³, Stephanie Rouse⁴, Kimberly Ramirez⁵, Artur Indzhykulian³, Sharon Kujawa³, Andreas Eckhard³, Divya Chari³

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Background: We recently uncovered a strong genetic association between a subset of Meniere's disease (MD) patients – specifically those with endolymphatic sac underdevelopment (ES hypoplasia) – and X-linked hypophosphatemia (XLH), a rare, dominantly-inherited phosphate-wasting disorder caused by loss-of-function mutations in the PHEX gene. In our human cohort, we identified a male-specific distribution of MD among XLH patients. Building on this clinical link, we developed a Phex-deficient murine model that recapitulates the sex-specific distribution of hearing loss. In the murine model, female carriers with one mutant allele (heterozygous, Phex +/-) exhibit moderately elevated distortion product otoacoustic emission (DPOAE) and auditory brainstem response (ABR) thresholds, whereas male mice (hemizygous, Phex -/Y) and females lacking any functional PHEX (homozygous, Phex -/-) demonstrate absent DPOAEs and ABR thresholds ≥ 90 dB SPL. Here, we characterize this murine model to investigate the pathomechanisms that could underlie the inner ear dysfunction.

Methods: For histologic analysis, temporal bone specimens were embedded in methyl methacrylate resin (Technovit 9100) and sectioned in the mid-modiolar plane. Endolymphatic hydrops was quantified as a ratio of the scala media cross-sectional surface area (EH) to an “ideal EH,” which was calculated using a theoretical, non-distended Reissner’s membrane. Cochlear hair cells and synapses were quantified on cochlear whole mounts immunostained for markers of hair cell bodies (Myosin-VIIa), pre-synaptic ribbons (CtBP2), and post-synaptic glutamate receptors (GluA2). Endocochlear potentials (EPs) were measured at the basal turn via cochleostomy in anesthetized mice with cochleostomy location confirmed on histology.

Results: Hemizygous male mice (n=20) demonstrated histologic evidence of severe endolymphatic hydrops normalized to littermate controls ($194\% \pm 37.2\%$, $p < 0.001$). Heterozygous female mice (n=15) demonstrated minimal endolymphatic hydrops ($107\% \pm 17.8\%$, $p > 0.05$). Surprisingly, however, all mutant genotypes (i.e., hemizygous male, heterozygous/homozygous female mice) exhibited normal organ of Corti morphology and hair-cell and synapse counts relative to wildtype littermates. Preliminary EP recordings were similar in hemizygous males and heterozygous females compared to wildtype: 88.7 mV (hemizygous male); 84.7 mV (heterozygous female); 92.2 mV (wildtype).

Conclusions: Dramatically elevated DPOAE and ABR thresholds in hemizygous male mice are associated with more severe endolymphatic hydrops on histology. However, both male and female Phex-deficient mice demonstrated preserved hair cell and synapse anatomy and normal EPs, suggesting that alternative molecular pathways may underlie the inner ear phenotype. The Phex-deficient murine model, which closely mimics the sex-specific features of the human XLH-MD phenotype, offers a compelling opportunity to investigate these mechanisms. Ongoing studies will further investigate the pathologic changes downstream of PHEX loss, with the goal of unlocking the pathophysiologic mechanisms of hearing loss in MD.

Exploring Potential Factors Affecting Blood-Labyrinth Barrier Permeability In Cisplatin-Induced Ototoxicity

12:15 p.m. - 12:30 p.m.

Franz Gareza*¹, Cathy Yea Won Sung¹, Amanda Bonczkowski², Lisa Cunningham²

¹National Institute on Deafness and Other Communication Disorders, National Institutes of Health, ²National Institute on Deafness and Other Communication Disorders

Background: Cisplatin is a widely used chemotherapeutic drug that has significant ototoxic effects, leading to permanent hearing loss. It circulates systemically and crosses the blood-labyrinth barrier (BLB) located in the stria vascularis to enter the cochlea. The BLB regulates molecular entry into the cochlea via a network of capillaries whose endothelial cells are joined by tight junctions. These capillaries are also surrounded by perivascular macrophages (PVMs), which are known to contribute to regulation of BLB permeability. Our previous study showed that ablation of macrophages, including PVMs, resulted in decreased cisplatin levels in cochlear tissues, suggesting that macrophages may increase BLB permeability thereby promoting cisplatin entry into the cochlea. However, molecular mechanisms by which macrophages regulate cisplatin entry are unknown.

Guided by insights from the blood–brain barrier (BBB) literature, where vascular endothelial growth factor A (VEGF-A) is a well-established regulator of barrier permeability, we investigated whether VEGF-A plays a similar role in modulating BLB permeability and cisplatin entry into the cochlea. To test this, we examined VEGF-A expression in the cochlea using our clinically relevant mouse model of cisplatin-induced ototoxicity.

Methods: Mice were subjected to three cycles of cisplatin treatment, each cycle consisting of once-daily cisplatin injection for four days (Cisplatin timepoint) followed by a ten-day recovery period (Recovery timepoint). Single nucleus RNA sequencing (snRNA-seq) was performed on cochleae harvested at different timepoints following cisplatin treatment. Saline-treated mice served as controls. To validate the snRNA-seq results, RNAscope, a commercially available in-situ hybridization technique, was performed on cochlear sections from these timepoints to detect the expression of *Vegfa* and *Flt1* mRNA upon cisplatin exposure. We assessed timepoints at Control, Cycle 1 Cisplatin, Cycle 1 Recovery, Cycle 2 Recovery, and Cycle 3 Recovery.

Results: snRNA-seq data show that *Vegfa* is expressed in basal and marginal cells of the stria vascularis and is upregulated after Cycle 1 Recovery period. The transcript for the VEGF-A receptor, *Flt1*, is expressed in endothelial cells and is also upregulated after Cycle 1 Recovery period, suggesting that local ligand-receptor interaction may affect BLB permeability. To validate these findings, we performed RNAscope, which confirmed that *Vegfa* mRNA is expressed in the basal and marginal cells and is upregulated following cisplatin exposure. *Flt1* mRNA expression is reduced following cisplatin exposure but remained expressed across all timepoints.

Conclusions: The structural and functional similarities between BLB and BBB suggest that VEGF-A, potentially regulated by macrophages, may contribute to BLB breakdown in the cochlea. Both snRNA-seq and RNAscope data show that VEGF-A is expressed in the cells within the stria vascularis and upregulated following cisplatin exposure. Future studies will focus on determining whether macrophages regulate VEGF-A expression and whether this regulation contributes to cisplatin entry and accumulation in the cochlea.

Podium 17: From Cochlea to Cortex: Aging-Related Changes in Auditory Processing and Brain Health

10:30 a.m. - 12:30 p.m.

Ballroom B, Floor 3

Moderators: Amanda Lauer and Karen Castano-Gonzalez

Degraded Fidelity of Temporal Coding in Aging Auditory Cortices

10:30 a.m. - 10:45 a.m.

Yunru Chen^{*1}, Patrick O. Kanold²

¹*Johns Hopkins University School of Medicine*, ²*Johns Hopkins University*

Background: Aging-related hearing decline reflects both peripheral pathology and central processing deficits. While peripheral pathology involves damage of hair cells in the cochlea,

central deficits can occur regardless of the peripheral damages. It is prevalent in aging human and animals to have reduced accuracy of perception of complex sounds in daily life, which can result from the aging-related changes of spectrotemporal processing of sounds. As temporal integration especially of coincident-onset harmonic complexes is gated by inhibitory neurons, and since inhibitory processing is impaired in aging. Thus, we speculated that the fidelity of temporal encoding is impaired in the auditory cortex (ACtx).

Methods: To investigate this hypothesis, we performed in vivo two-photon imaging in CBA;Thy1-GC6s mice (young adult: 3-6 mo; aging: 20-24 mo) to investigate how aging ACtx respond to temporal shifts in frequency of two-tone harmonic sounds compared to young adults. We recorded calcium signals in primary ACtx (A1) layer (L) 2/3, A1 L4, and L2/3 of secondary ACtx (A2). We presented two-tone harmonic stacks (base frequency $F_0 = 4\text{-}16\text{kHz}$) under temporal shifts (higher tone onset ± 15 or ± 30 ms relative to F_0). Neurons were classified from responses to pure tones and harmonic stacks as pure-tone sensitive (PTN), harmonic-sensitive (HN), and both-sensitive (BN).

Results: Across regions, aging altered the composition and sensitivity of responsive populations. In L2/3 of A1 and A2, the proportion of BN increased, while HN decreased in A2 L2/3, consistent with reduced stimulus specificity to pure tones or harmonics and greater mixing of tuning in the superficial layers of aging mice. With temporal shifts of 15ms and 30ms, all imaged subregions showed more neurons responding in aging than in young mice, indicating a broadened temporal window for multi-frequency sounds and reduced temporal sensitivity for coincident onsets.

Conclusions: These results show that aging preserves the prevalence of harmonic sensitivity in some circuits but degrades the fidelity of harmonic coding in that all imaged areas show diminished temporal precision (more response to shifted onsets). We speculate that these changes in temporal encoding fidelity underlie some of the hearing deficits in older individuals.

Aspirin Use and the Rate of Hearing Decline in Aging: Results From a Longitudinal Cohort Study

10:45 a.m. - 11:00 a.m.

Lauren Dillard^{*1}, Lois Matthews¹, Jada Johnson¹, Judy Dubno¹

¹*Medical University of South Carolina, College of Medicine*

Background: Research regarding the effects of aspirin on the auditory system is equivocal. Some studies suggest it can have deleterious ototoxic effects, whereas others suggest it may be protective against hearing loss. Most epidemiological studies evaluating relationships between aspirin use and hearing among the general population are cross-sectional, although longitudinal data are needed to understand the temporality of associations. The purpose of this study, conducted in a community-based cohort study of the general population, was to determine the association of aspirin use with the rate of hearing change per year.

Methods: Participants were adults from the Medical University of South Carolina Longitudinal Cohort Study of Age-related Hearing Loss, an ongoing (1988-current) community-based cohort study based in Charleston, SC. Use of medications, including aspirin, was self-reported at participants' baseline examination. Participants underwent pure-tone audiometric testing at the

baseline examination and at annual follow-up examinations. We defined hearing by a pure-tone average (PTA) of thresholds at 0.5, 1.0, 2.0 and 4.0 kHz, averaged bilaterally. We used multivariable-adjusted linear mixed regression models to estimate the effect of age (per year) on the rate of PTA change for participants who did and did not report aspirin use at baseline, adjusted for baseline age, sex, and race. The effect of aspirin use on the rate of annual PTA change was determined by an interaction term of age (longitudinal time variable) and aspirin use. A sensitivity analysis excluded participants reporting taking other anti-inflammatory medications.

Results: This study included 1203 participants, with a mean baseline age of 64.1 (SD 14.1) years, PTA of 23.8 (SD 15.0) dB HL, and follow-up time of 4.8 (SD 5.6) years; 59.8% were female and 18.3% racial Minority. The prevalence of aspirin use at baseline was 27.1%. Participants who reported aspirin use had a similar baseline PTA (23.8 dB HL) to those who did not (23.8 dB HL), with a mean difference of -0.02 dB between groups ($p=0.98$). In multivariable-adjusted linear mixed effects models, aspirin use was associated with a higher ($p < 0.01$) rate of decline per year (0.96 dB), compared to no aspirin use (0.81 dB). Results were similar in a sensitivity analysis excluding participants ($n=185$) taking other anti-inflammatory medications.

Conclusions: Aspirin use was not associated with baseline hearing, but it was associated with a higher rate of annual decline to hearing. However, the association was relatively small, equating to an approximate difference of 1 dB over 10 years. Planned analyses will evaluate whether (1) aspirin use is associated with changes to hearing across the frequency range and (2) associations vary by (non-)continuous use of aspirin over time and/or by dose. Longitudinal studies are needed to determine the impacts of aspirin use, and its dosing, on changes to hearing over time.

DNA Methylation Analysis Identifies the Role of TNFRSF25 in Age Related Hearing Loss

11:00 a.m. - 11:15 a.m.

Pei-Ciao Tang^{*1}, Marie Valerie Roche¹, Denise Yan¹, Michelle De Marchena¹, Maria Camila Robayo¹, Feng Gong¹, Yan Guo², Katherina Walz³, Xue Liu¹

¹*University of Miami School of Medicine*, ²*University of Miami Miller School of Medicine*,

³*University of Miami School of Medicine, John P. Hussman Institute for Human Genomics; John T Macdonald Foundation*

Background: Age-related hearing loss (ARHL), also called presbycusis, is a common form of sensorineural hearing loss in older adults. Epigenetic mechanisms regulate gene expression without altering the DNA sequence and include DNA methylation, histone modifications, ATP-dependent chromatin remodeling, and noncoding RNAs. Specifically, DNA methylation is a process of adding a methyl group to the CpG site. Methylation at promoters and transcription start sites (TSSs) generally represses transcription, whereas lack of methylation is typically permissive. CpG methylation contributes to the development and function of the cochlear sensory epithelium, and aberrant methylation patterns have been linked to ARHL. We hypothesized that ARHL patients exhibit different methylation levels in genes compared to the control group.

Methods: DNA from human subjects with and without ARHL underwent array-based methylation profiling using the Illumina MethylationEPIC BeadChip. Linear and logistic regression were used to assess the relationship between hearing threshold- as binary or continuous, respectively- and CpG-site M-values in 14 ARHL patients, with adjustments for age, sex, and race. Candidate genes demonstrating significantly different methylation profiles vs. control patients were identified, with animal models were used to confirm certain findings.

Results: Linear regression revealed robust associations between CpG sites in ESPN and TNFRSF25 and hearing thresholds among the ARHL patients. To investigate the role of the latter, we generated a Tnfrsf25-knockout (KO) mouse model, and an auditory brainstem response (ABR) test showed that KO animals exhibited hearing loss, with thresholds that worsened with age. Loss of hair cells and neural innervation was observed in 8-month-old KO mice. We also assessed expression of other candidate genes associated with CpG sites with significantly different methylation between ARHL patients and controls in the mouse cochlea. We observed the expression of Dnmt3a and Uqcr11 in the mouse cochlea.

Conclusions: By examining CpG methylation, this study identified genes associated with ARHL. The role of TNFRSF25 in hearing was supported by results from Tnfrsf25-KO mice. Expression patterns of additional candidates, including Dnmt3a, in the mouse cochlea support the feasibility of this model for future studies on DNA methylation and ARHL.

AI-Based Assessment of Auditory Evoked Cortical Potential Efficiently to Detect Alzheimer's Disease

11:15 a.m. - 11:30 a.m.

Imran Iqbal^{*1}, Chun Liang¹, Tian-Ying Zhai¹, Zhi-Rui Zhao¹, Hong-Bo Zhao¹

¹*Yale University Medical School*

Background: Alzheimer's disease (AD) is a progressive neurodegeneration disease, starting from synaptic dysfunction to eventually neuronal degeneration. Early detection and diagnosis are important for AD prevention and treatment but remain big challenges; there are no reliable and easily practicable methods available to diagnose AD development and progression. Our previous study (Mei et al., 2021, Front Aging Neurosci. 13;13:710317.) demonstrated that the auditory-evoked cortical potential (AECp) is sensitive to dysfunction in the central auditory system and other brain areas as well and could capture AD related changes. However, AECp is very complex and hard to identify and assess its changes by regular and traditional analyses.

Methods: APP/PS1 transgenic AD mice and littermate wild-type (WT) mice were used. As reported in our previous paper (Mei et al., 2021), AECps were recorded from 192 mice (WT = 91, APP = 101) at 11 ages (1–10, 12 months) with three stimuli: 16 kHz tone burst, click at 85 dB SPL, and click at 95 dB SPL. Domain features, such as amplitudes and latencies of Waves I–V, inter-peak intervals I–III and III–V, signal energy, and envelope variance, were extracted. The convolutional neural network (CNN) was trained with Barlow Twins on per-channel z-scored waveforms using morphology-preserving augmentations. After per-feature standardization, we applied supervised UMAP (Uniform Manifold Approximation and Projection) to visualize three tasks: genotype (WT vs APP), age (1–10, 12 months), and genotype × age. Cluster quality was quantified by the silhouette score. To aid interpretation, we computed permutation-based features for the domain features.

Results: Domain features yielded moderate structure: silhouette 0.60 (genotype), 0.52 (age), and 0.48 (genotype $\times$ age). Hybrid features markedly improved separation to 0.92, 0.85, and 0.77 for the same tasks, respectively; the overall summary silhouette increased from 0.60 (domain) to 0.92 (hybrid). UMAP embeddings showed two compact, non-overlapping genotype regions. The difference could be early detected at 1 month old. The 95 dB SPL click contributed strongly to separation, particularly for later-wave latency and III–V interval features, patterns consistent with slowed neural conduction and reduced response robustness in APP mice with aging. Domain-feature permutation highlighted envelope variance, signal energy, Wave I latency, and III–V interval, features that plausibly track synchrony loss and conduction delay in AD. The hybrid gains indicate that the SSL (self-supervised learning) embeddings add complementary, distributed waveform information beyond these audiology-informed markers.

Conclusions: AI-based AECF assessment can efficiently detect early brain functional changes in AD and assess AD development and progression. Such AI-based assessment of AECF may also be used in other hearing disorders, such as age-related hearing loss and tinnitus, to assess cortex functional changes.

Supported by NIH AF1 AG082216 and R01AG080587 to HBZ

Age-Dependent Loss of Temporal Precision in Inferior Colliculus Neurons of Unanesthetized Mice

11:30 a.m. - 11:45 a.m.

Chun-Jen Hsiao^{*1}, Nicholas Giorgio¹, Alexander Galazyuk¹

¹*Northeast Ohio Medical University*

Background: Humans and animals rely on complex sound sequences for communication. In humans, speech perception depends on the auditory system's ability to detect rapid changes in sound level and frequency over time scales ranging from hundreds to tens of milliseconds. Older adults often experience difficulty understanding speech despite having relatively normal hearing thresholds. Compared with younger adults, they show reduced sensitivity to rapid but not slow, temporal fluctuations in speech. These findings highlight the need to examine central auditory temporal acuity across both short and long intervals throughout the lifespan. Mice, whose vocalizations incorporate intervals as short as ~20 ms, provide a suitable model for investigating these temporal processing mechanisms.

Methods: Extracellular recordings were obtained from inferior colliculus (IC) neurons in unanesthetized CBA/CaJ mice aged 2, 12, and 24 months using glass microelectrodes. Neuronal responses were recorded to sequences of 20-ms pure tones spanning 2,346 frequency–level combinations (2–60 kHz in 0.1-octave steps; –10 to 80 dB SPL in 2-dB steps), presented in pseudo-random order. Each IC neuron was tested with four identical sequences delivered at inter-stimulus intervals (ISIs) of 250, 100, 50, and 20 ms. Frequency response areas (FRAs) were reconstructed, and mean firing rate and first-spike latency variance (jitter) were compared across conditions.

Results: Across all age groups, most IC neurons exhibited similar FRA shapes across ISIs. However, firing rate and first-spike latency variance were strongly influenced by ISI and age. In

2-month-old mice, shorter ISIs increased firing rate and reduced first-spike latency variance, indicating enhanced temporal acuity. In contrast, in 12-month-old mice—and more prominently in 24-month-old mice—shorter ISIs reduced firing rates and increased first-spike latency variance. These findings demonstrate an age-related decline in temporal acuity for short ISIs.

Conclusions: Short ISIs enhance both response strength and temporal precision in young animals, consistent with an improved signal-to-noise ratio and greater temporal acuity. This rate-dependent enhancement diminishes with age, highlighting a decline in auditory temporal processing during aging.

This study was supported by NIH R01 DC016918 from the National Institute on Deafness and Other Communication Disorders.

Abnormal Hearing Phenotypes in Knockout Mice as Predictors of Cognitive Dysfunction

11:45 a.m. - 12:00 p.m.

Sergio Vicencio-Jimenez¹, Micheal Dent², Amanda Lauer^{*3}

¹*Johns Hopkins University School of Medicine*, ²*University at Buffalo, SUNY*, ³*Johns Hopkins University*

Background: Alzheimer's disease and related dementias (ADRD) affect millions worldwide, placing a heavy burden on individuals, families, and healthcare systems. Age-related hearing loss is one of the most prevalent predictors of ADRD risk, offering potential for early intervention. While the causal relationship between hearing loss and ADRDs remains uncertain, and most ADRD mouse models focus on rare familial mutations, limiting their ability to capture the broader genetic and phenotypic variability of late-onset ADRD. Our study used large-scale knockout mouse data from the International Mouse Phenotyping Consortium to investigate sensory–cognitive/emotional relationships and identify novel genetic candidates and mechanistic pathways that may link hearing loss and ADRDs.

Methods: Phenotypic data from more than 9000 knockout lines were analyzed to assess whether auditory dysfunction co-occurred with behavioral abnormalities, particularly in cognition and emotion. Severity of hearing loss was quantified using auditory brainstem response (ABR) thresholds, and Gene Ontology enrichment analyses were performed on genes linked to auditory, cognitive, emotional, and general behavioral phenotypes.

Results: Gene knockout strains with abnormal auditory phenotypes were significantly more likely to show abnormal behaviors, including cognitive and emotional deficits. While other sensory modalities, such as vision, also showed associations with abnormal behaviors, auditory dysfunction exhibited more robust patterns. Elevated ABR thresholds were positively correlated with the number of abnormal behaviors. Gene Ontology analyses revealed several biological processes linking auditory and cognitive-emotional dysfunction, including pathways related to synaptic signaling and cellular regulation.

Conclusions: Our results point to candidate genes that may link auditory and cognitive-emotional phenotypes. Importantly, hearing loss emerged as a particularly sensitive predictor of behavioral abnormalities, supporting its potential as a biomarker and a target for improving ADRD models.

Age-Related Hearing Loss and Inflammatory Profile in the Human Aged Cochlea

12:00 p.m. - 12:15 p.m.

Maria Luque^{*1}, Rudolf Glueckert¹, Marta Konopka¹, Pedram Ghaderi¹, Erich Brenner¹, Marko Kanschake¹, Elisabeth Pechriggl¹

¹*Medical University Innsbruck*

Background: Age-related hearing loss (ARHL) is among the most prevalent manifestations of ageing. According to the World Health Organization, hearing loss affects ~30% of individuals over 60 years, and by 2050 one in ten people is projected to suffer from disabling hearing loss. Beyond communication impairment, ARHL accelerates cognitive decline and imposes a substantial burden on healthcare systems. Chronic low-grade inflammation, commonly referred to as “inflammaging,” has emerged as a key contributor to sensorineural hearing loss alongside environmental insults such as noise exposure. A potential player in this process may be the nucleotide-binding domain, leucine-rich repeat, and pyrin domain-containing protein 3 (NLRP3) inflammasome, a cytosolic multiprotein complex that mediates the release of pro-inflammatory cytokines, including IL-1 β and IL-18. NLRP3 activation has been implicated in neurodegenerative and cardiovascular diseases, and recent evidence links it to auditory dysfunction.

This study investigates age-related inflammatory responses in rodent models of hearing loss and human cochlear tissue, with a focus on NLRP3 inflammasome activation and its correlation with auditory decline.

Methods: Immunohistochemistry was employed to examine immune activation and inflammatory markers in rodent cochleae of different ages. These findings were systematically compared with human cochlear specimens from elderly donors. Quantitative analyses assessed microglial activation, macrophage distribution, and their association with auditory brainstem response (ABR) thresholds in rodents and spiral ganglion neuron (SGN) integrity in humans.

Results: Inflammatory activity increased with age in both rodent and human cochleae. Microglial activation patterns showed a significant correlation with ABR thresholds in rodents, while in human samples macrophage distribution was closely associated with SGN degeneration. These findings indicate a conserved age-dependent inflammatory phenotype across species.

Conclusions: Characterizing the role of inflammation in cochlear ageing advances our understanding of ARHL pathophysiology. Elucidating NLRP3-mediated mechanisms may reveal novel therapeutic targets to mitigate auditory decline and its broader neurocognitive consequences in ageing populations.

Proteomic Profiling of Age-Related Hearing Loss in Mouse Cochleae Using Laser Microdissection and LC-MS/MS

12:15 p.m. - 12:30 p.m.

Norio Yamamoto*¹, Maiko Okamura², Takashi Nirasawa³, Kengo Yoshii⁴, Masaya Ikegawa², Yoko Fukunaga⁵, Hiroe Ohnishi⁵

¹Kobe City Medical Center General Hospital, ²Faculty of Life and Medical Sciences, Doshisha University, ³Bruker Japan K.K., ⁴Kyoto Prefectural University of Medicine, ⁵Graduate School of Medicine, Kyoto University

Background: Age-related hearing loss (ARHL) is the most prevalent form of sensorineural hearing impairment and remains intractable due to the limited regenerative capacity of the inner ear. Given its association with cognitive decline and the increasing aging population in developed countries, elucidating the molecular mechanisms underlying ARHL is critical for developing preventive and therapeutic strategies. Recent advances in omics technologies have enabled comprehensive profiling of proteins alongside mRNAs. In this study, we performed in-depth proteomic analyses of cochlear tissues from an ARHL mouse model to identify age-dependent changes in protein expression.

Methods: Cochlear sections were prepared without fixation from C57BL/6 mice aged 6 weeks, 6 months, and 12 months. Laser microdissection was employed to isolate the organs of Corti, stria vascularis, and cochlear nerve. Proteins were extracted, digested with trypsin, and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using the timsTOF Pro system (Bruker). Shotgun proteomics was used to identify inner ear-specific and age-variable proteins. Relative protein abundance was quantified using the Normalized Spectral Abundance Factor (NSAF), with a > 1.5-fold change considered significant. Gene ontology analysis was conducted using DAVID to assess biological functions and relevance to ARHL pathogenesis.

Results: LC-MS/MS analysis identified approximately 1000–2000 peptides and 100–300 proteins per sample. Antioxidant-related proteins were upregulated in the stria vascularis between 6 and 12 months of age. Conversely, proteins involved in protein refolding were downregulated in the organs of Corti and cochlear nerve over the same period.

Conclusions: Our findings demonstrate that proteomic profiling of cochlear tissues can reveal age-dependent molecular changes associated with ARHL. These insights may facilitate the identification of key pathogenic proteins and support the development of novel therapeutic approaches for ARHL.

Symposium 5: Vestibular Contributions to Cognitive Decline: From Inner Ear Trauma to Aging

10:30 a.m. - 12:30 p.m.

Meeting Room 208ABC, Floor 2

Vestibular Contributions to Cognitive Decline: From Inner Ear Trauma to Aging

Avril Genene Holt, *Wayne State University School of Medicine*

Symposium Description: The vestibular system is essential for balance and spatial orientation but also for higher-order cognitive functions such as spatial memory, navigation, and executive control. A growing body of evidence links vestibular dysfunction due to aging, trauma or environmental exposures to cognitive decline and impaired spatial processing. This emerging field highlights the vestibular system as both a marker and a modifiable contributor to cognitive health across the lifespan. This symposium brings together leading and emerging researchers to synthesize current understanding of how vestibular dysfunction contributes to cognitive decline and to explore translational opportunities for diagnosis and intervention. Talks will span basic mechanisms, human studies, and the important role of novel technologies. Dr. Daniel Merfeld (Ohio State University) will examine relationships between aging, vestibular perceptual thresholds, balance, and fall risk. Dr. Nora Fritz (Wayne State University) will share neuroimaging and behavioral data linking vestibular and cognitive function. Dr. Richard Altschuler (University of Michigan) will explore how mTOR pathway modulation affects noise-induced and age-related vestibular dysfunction. Dr. Federica Raciti (University of Miami) will describe a multimodal translational framework for assessing vestibular dysfunction after trauma. Dr. Christopher Pastras (Macquarie University, Australia) will introduce innovative vestibular assessment techniques revealing mechanisms of balance transduction in health and disease. Danial Naqvi (Wayne State University) will discuss how blast-induced vestibular dysfunction creates progressive neural synchrony changes and intensity coding deficits that may contribute to cognitive impairment. Finally, Celia Fernandez Brillet (Johns Hopkins University) will present findings on how vestibular prosthetic stimulation improves dual-tasking in aging adults. Together, these presentations will illuminate shared mechanisms and novel strategies for early identification and treatment. This timely, multidisciplinary symposium addresses a critical and underexplored aspect of cognitive health by bringing together leading researchers to present cutting-edge findings on the vestibular-cognitive interface and discuss translational approaches to address this critical public health issue.

Suhrud Rajguru, *University of Miami*

Relationships Between Aging, Vestibular Perceptual Thresholds, Balance, and Fall Risk

Daniel Merfeld, *Ohio State University*

Individual Abstract: It has long been known that the risk of falling increases with age and that balance control ability also declines with age. These age-related balance declines have been shown to correlate strongly with self-reported falls (Agrawal et al, 2009). Data also show that vestibular perceptual thresholds vary with age; thresholds remain relatively constant below the age of 40 and then increase monotonically with age (Bermúdez Rey et al, 2016). One of these perceptual thresholds – the one measured using 0.2 Hz roll tilt stimuli – has been shown to correlate with balance performance (Karmali et al, 2017). A later mediation analysis of these same data showed that nearly 50% of the effect of age on balance performance could be explained by the perceptual roll tilt thresholds (Beylergil et al., 2019). Other studies have shown that roll tilt thresholds can be improved with extensive training (Klaus et al, 2020). Later vestibular perceptual learning studies showed that balance improves when exposed to a perceptual learning protocol in both young adults (Wagner et al, 2022) and older adults (Fitze et al, 2024). These findings highlight the contributions of aging to vestibular function and balance but also show that simple perceptual learning protocols may help reduce fall risk.

A Balancing Act: Sensorimotor, Cognitive, and Neuroimaging Insights Into Falls in Neurodegenerative Disease

Nora Fritz, *Wayne State University*

Individual Abstract: Background: Falls are an unfortunate commonality among individuals with neurodegenerative diseases, with more than 70% of individuals with multiple sclerosis (MS) alone reporting falls in a 6-month period. While mobility, cognition, and sensory function deficits have been identified as contributing factors, the complex interplay between neural structure, function, and clinical symptoms remains poorly understood.

Objectives: This session will present our lab's latest research, shedding light on the critical role of sensory reweighting (including both vestibular and proprioceptive reliance), cognitive function, and advanced structural and functional MRI metrics in predicting falls and concern about falling.

Results: We have shown that backward walking (BW) is a sensitive indicator of fall risk in persons with MS; indeed the impact of disease severity on BW performance is significantly mediated by cognition. Further, we have demonstrated cerebellar contributions to mobility, cognition and proprioception in persons with MS and identified differences in cerebellar volumes between MS fallers and non-fallers. Finally, our newest work demonstrates changes in functional connectivity between amygdala—cerebellum and amygdala-hippocampus in individuals with MS who endorse greater concerns about falling.

Conclusions: Measurement of multiple systems, including cognition, motor, and sensory systems is needed to better understand falls in MS. Future work will incorporate these findings into intervention design to ultimately reduce fall risk and improve patient outcomes.

Blast-Induced Vestibular Dysfunction: Evolving Neural Synchrony and Intensity Coding Deficits With Implications for Cognitive Performance

Danial Naqvi, *Wayne State University School of Medicine*

Individual Abstract: Blast exposure (no tympanic membrane rupture) represents a significant yet understudied form of trauma that causes temporary auditory threshold shifts while generating complex vestibular deficits. Understanding the temporal evolution of these vestibular changes is critical for developing targeted interventions and improving clinical outcomes in blast-exposed populations. We report longitudinal vestibular short latency evoked potential (VsEP) changes in a preclinical blast model that reveal a progressive pattern of neural dysfunction and recovery.

During the acute phase immediately following blast exposure, VsEP waveforms are significantly diminished, indicating a profound loss of synchronous vestibular nerve activity that disrupts normal neural transmission. By day 7 post-exposure, waveforms appear to normalize, suggesting that the initial disruption represents a transient but severe functional impairment rather than permanent structural damage. However, by day 20, a more complex pattern emerges where early VsEP waves return to baseline parameters, but later waves exhibit significantly increased amplitudes, suggesting a type of neural hyper-synchrony that may reflect maladaptive plasticity.

Perhaps most significantly, blast exposure abolishes the normal amplitude scaling response to varying jerk stimulus intensities, fundamentally disrupting intensity coding mechanisms. While

high-intensity stimuli at 2,500 g/s continue to elicit measurable responses, the vestibular system fails to respond appropriately to lower-intensity 500 g/s stimuli, indicating severely impaired intensity discrimination capabilities. This disrupted intensity coding may degrade spatial orientation and navigation abilities, potentially contributing to cognitive dysfunction through aberrant vestibular input to forebrain and cortical networks. If parallel behavioral assessments demonstrate correlated changes in spatial memory and motor coordination, they will provide critical mechanistic links between peripheral vestibular dysfunction and cognitive impairment. This work suggests that blast-induced vestibular dysfunction may be a key contributor to neural processing errors leading to cognitive decline.

New Frontiers in Vestibular Assessment: Revealing Mechanisms of Balance Transduction in Health and Disease

Christopher Pastras, *Macquarie University*

Individual Abstract: Accurately quantifying peripheral vestibular function in vivo remains a significant challenge due to the anatomical inaccessibility of the labyrinth and its proximity to the cochlea. To address this, we have developed a suite of novel in vivo electrophysiological and mechanical techniques to differentially probe vestibular hair cell function, afferent nerve signaling, and organ-level mechanics in anaesthetized rodent models. Our approach combines vestibular

microphonic and summating potential recordings to assess hair cell activity, alongside compound action potentials and vestibular neurophonics to evaluate afferent sensitivity and synchrony.

Additionally, we have adapted Laser Doppler Vibrometry and Optical Coherence Tomography to quantify utricular mechanics in vivo. Using this integrated platform, we have identified key features of signal transmission between Type I hair cells and their afferent calyces, demonstrating that

ultrafast nonquantal synaptic transmission underlies the exceptionally short-latency, high-fidelity responses of vestibular afferents to transient stimuli. Ongoing work applies these tools to investigate how vestibular function and mechanics are altered by experimental perturbations relevant to disease states, and how electrical and optical stimulation strategies can be optimized for targeted neuromodulation. These findings provide a new framework for understanding peripheral vestibular encoding and its role in health, aging, and balance disorders.

Capturing Vestibular Dysfunction After Trauma: A Multimodal and Translational Framework for Preclinical Testing

Federica M. Raciti, *University of Miami*

Individual Abstract: Emerging evidence indicates that vestibular dysfunction plays a critical role in long-term impairments in spatial orientation, balance, and mobility, and may also contribute to cognitive decline, particularly in aging populations and individuals affected by environmental trauma. Despite its clinical significance, progress in understanding, diagnosing, and treating vestibular dysfunction has been limited by multiple factors, including the

complexity of vestibular pathways and the lack of preclinical tools that replicate the sensitivity of clinical tests. Many existing preclinical assessments cannot capture subtle or end-organ-specific functional deficits, limiting translational relevance and mechanistic insight. To address this gap, we developed a multimodal preclinical test battery designed to detect vestibular dysfunction with high specificity and temporal resolution. The platform integrates electrophysiological, behavioral, and morphometric approaches to assess peripheral vestibular function at both acute timepoints and longitudinally. It incorporates preclinical adaptations of clinical vestibular tests commonly used in human diagnostics, enhancing alignment with clinical outcome measures and improving translational applicability. Using ecologically relevant models of noise and blast overexposure, we identified distinct patterns of acute and persistent vestibular deficits, including subclinical changes in balance and vestibular reflexes. By enabling the detection of both overt and subtle impairments, this framework offers an informative approach to characterize vestibular injury, monitor recovery, and support therapeutic development. It serves as a critical translational bridge between animal models and clinical application and provides a foundational tool for future investigations into vestibular pathophysiology and repair.

The Influence mTOR Pathway Modulation on Age-Related and Noise-Induced Hearing Loss and Vestibular Dysfunction

Richard Altschuler, *University of Michigan*

Individual Abstract: Rapamycin acting on mammalian target of rapamycin (mTOR) pathways has been shown to increase longevity in animal models and enhance cell survival following different types of stress across species and body regions. We have found that adding rapamycin to diet will reduce / decelerate age-related hearing loss in the mouse model (Altschuler et al. 2018,2021). We also found adding rapamycin to diet before or shortly after noise overstimulation reduces auditory brain stem response threshold shifts, both temporary and permanent (depending on noise exposure conditions), consistent with studies showing intraperitoneal delivery of rapamycin reduces noise-induced hearing loss (Yuan et al., 2015). We are currently examining the influence of rapamycin treatment on age-related vestibular dysfunction and noise-induced vestibular dysfunction. Our preliminary results show adding rapamycin to diet reduces noise-induced vestibular decrements in balance beam crossing and calretinin immunostaining of calyces in the rat model. While rapamycin can have side-effects with long-term human application, “rapalogs” acting on mTORC1 or 2 pathways are being developed with fewer side-effects. Since hearing loss and vestibular dysfunction both contribute to cognitive decrements, modulation of mTOR functional signaling pathways and their downstream targets could play an important role in reducing this decline.

Prosthetic Vestibular Stimulation Improves Dual-Tasking Performance in Aging Populations

Celia Fernandez Brillet, *Johns Hopkins University*

Individual Abstract: Background: Bilateral vestibular hypofunction (BVH) impairs gaze- and spinal-stabilizing reflexes. Individuals with BVH frequently report experiencing increased mental fog and challenges with dual-tasking while walking, such as when attempting to walk and converse simultaneously. Although the interaction between age and dual-tasking capabilities

following vestibular implantation is not well understood, it is an important factor in the impact of vestibular prosthetic inputs on activities of daily living.

Methods: We followed 11 adults up to one year after unilateral vestibular implantation for bilateral vestibular hypofunction. Our tested groups consisted of a younger cohort (n = 5, median age 55, range 45–60 at the time of implantation) and an older cohort (n = 6, median age 71, range 69–80 at the time of implantation). To assess the effects of cognitive load on walking tasks, we administered Timed Up and Go (TUG) with and without cognitive distractions (Serial Sevens downward counting). Following device activation, we tested patients undergoing prosthetic stimulation using two protocols: motion-modulated and tonic (constant rate and amplitude) stimulation.

Results: Preoperatively, within-participant TUG times averaged across patients (mean±SD) varied between age groups, equaling 11.1±2.5 s for the younger cohort and 18.5±4.1 s for the older cohort. Adding the Serial Sevens cognitive task increased (worsened) preoperative TUG scores to 12.8±2.9 s for the younger cohort and 21.6±4.2 s for the older cohort. At the most recent post-implantation visit, the younger cohort's within-participant TUG times had improved by 3.6±2.3 s during motion-modulated stimulation and by 3.0±1.3 s during tonic stimulation. Performance during dual-tasking (Serial Sevens and TUG) improved by 4.8±2.2 s with motion-modulated stimulation and by 4.0±1.1 s with constant stimulation. For the older cohort, TUG times improved by 4.3±3.6 s and 3.6±3.0 s for motion-modulated and constant stimulation, respectively. Dual-tasking scores improved by 5.2±4.1 s and 5.5±4.0 s for motion-modulated and constant stimulation, respectively.

Conclusions: Compared to younger patients, older patients take longer and are more affected by dual-tasking while completing the TUG test. Throughout the first year of device activation, prosthetic stimulation delivered by a unilateral vestibular implant resulted in improved performance in both cohorts during the TUG and TUG + serial sevens tasks.

Support: U01DC019364, R01AG076701, Rafael del Pino Foundation, “la Caixa” Foundation

Poster Session IV with Coffee

1:30 p.m. - 3:00 p.m.

Ballroom A, Floor 3

Due to the policies of the Puerto Rico Convention Center, the Poster Hall will be available to attendees between the hours of 7AM – 11PM (local time), with a 1- hour break every day between 11AM and 12 PM (Noon) to facilitate setup for the next poster session. This is an appreciated extension of their normal permitted hours. ePosters are available on the conference website and the ARO MWM mobile app. and nbsp;

T1. Mitochondria-Related Protein Aggregation in Bushy Cells of Cochlear Nucleus During Aging

Meijian Wang*¹, Haochen Ding², Ruijie Cai³, Ting Zhao³, Xiaotong Ma³, Mei Shan³, Weijun Zhou³, Yunge Gao³, Jun Xu³, Xintong Li², Liping Zhang², Huihui Liu³, Ben Loos⁴, Hao Wu³

¹*Shanghai Jiao Tong University, School of Medicine*, ²*College of Health Science and Technology, Shanghai Jiao Tong University School of Medicine, Shanghai, China*, ³*Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China*, ⁴*Stellenbosch University, Stellenbosch, South Africa*

Background: This study follows upon our previous presentations at ARO meetings regarding the proteomic analysis of the cochlear nucleus (CN) in age-related hearing loss (ARHL)—one of the most prevalent health conditions affecting older adults. In our prior work, we illustrated aging-related proteomic alteration atlas in the CN; among which significantly changed proteins were enriched in mitochondria-related proteins. Notably, our current investigation reveals that the upregulated proteins during aging exhibit a tendency to aggregate in bushy neurons—a population of energy-demanding neurons within the CN.

Methods: CN cryosections were prepared from C57BL/6J mice of three age groups: young (~3 months), mid-age (~10 months), and old (~15 months). All the 11 mitochondria-related proteins identified with significant abundance changes during aging in our previous proteomic analysis (9 with increased abundance, 2 with decreased abundance) by the comparisons between old vs. young CN: 8 proteins (6 with increased abundance, 2 with decreased abundance); old vs. mid-age CN: 7 proteins (6 with increased abundance, 1 with decreased abundance; 4 of which overlapped between the two comparison), were examined by immunostaining with their respective antibodies in the current study. Additionally, we used antibodies against Tomm20 and VGlut1, which are widely accepted markers for mitochondria and bushy neurons, respectively, and these two markers were simultaneously stained with each of the 11 target proteins. After confocal microscopy imaging of the immunostained markers, the same sections were demounted and processed for transmission electron microscopy (TEM). Both confocal images (visualizing mitochondria-related protein distribution and mitochondrial morphology) and TEM micrographs (providing high-resolution details of mitochondrial structure) were analyzed to cross-validate findings across imaging modalities.

Results: Our preliminary data showed that at least 5 out of the 9 mitochondria-related proteins with increased abundance tend to aggregate into more patches in bushy neurons of the old age group than in those of the younger groups. The mitochondria that overlapped with these aggregated proteins were larger in volume—compared to those that did not overlap with the aggregated proteins—and this larger volume might indicate reduced mitochondrial vitality. This (i.e., larger volume of mitochondria overlapping with aggregated proteins) was observed not only in the old age group but also in the younger groups (occasionally, young bushy cells appear these protein aggregating patches with smaller size).

Conclusions: The current findings reveal that mitochondria-related proteins undergo aggregation in bushy neurons during aging. This phenomenon not only suggests that such aggregation may serve as a potential driving mechanism for age-related degradation in these high-energy-demanding cells but also provides a potential target for intervening in age-associated impairments involving these neurons.

T2. Ergothioneine Attenuates Age-Related Hearing Loss Through Ergothioneine Transporter and Sex Hormone Crosstalk

Bo Ding^{*1}, Xiaoxia Zhu², Robert Frisina²

¹University of South Florida, ²Global Ctr. Hearing and Speech Res., University of South Florida

Background: Previous analyses of auditory brainstem response (ABR) threshold shifts in old (30 month old) CBA/CaJ mice indicated that ergothioneine (EGT) treatment exerts greater protective effects in males compared to females. These findings raised the question of whether ergothioneine (EGT) protective effects have sex-specific efficacy. The present study investigates relationships between sex hormone levels and the expression of the EGT transporter, ergothioneine transporter (ETT), to elucidate potential mechanisms underlying sex-dependent responses to EGT therapies.

Methods: In vivo experiments employed middle-aged male and female CBA/CaJ mice (15 months old, N=6 per sex) who were orally treated with EGT via gavage for 16 weeks. The mouse auditory function was evaluated using ABRs. In parallel, an in vitro cultured cochlear cell line was employed using HEI-OC1 cells, which were treated with estradiol (E2) at the concentrations of 10nM or testosterone (T) 100nM for 24 hours. EGT levels and ETT protein expression were detected using anti-EGT polyclonal antibody through immunohistochemistry and western blots. ETT gene expression was determined by quantitative RT-PCR.

Results: In middle age female mice, ABR thresholds rose with age when EGT was not administered (Controls), reflecting an age-related decline in hearing ability. However, EGT supplementation mitigated this age-associated loss. In male mice, ABR thresholds showed minimal change over time, independent of EGT exposure. In contrast to our earlier observation that EGT conferred greater auditory protection in very old males than in females (30-month-old CBA/CaJ mice), the present findings indicate that its protective effect is more pronounced in premenopausal females than in their aged counterparts. Compared to controls, significantly greater levels of EGT were detected in the cochlea, organ of Corti, spiral ganglion cells, and stria vascularis in EGT treated groups, demonstrating that EGT penetrates the blood-cochlear labyrinth barrier. Interestingly, we did not find significant changes in ETT mRNA levels due to E2. Similarly, we observed no ETT mRNA expression difference in mouse inner ear tissues treated with EGT compared to non-treatment groups or young adult cochlea vs. old cochlea. ETT protein expression was decreased in old spiral ganglion neurons compared with young adults. Immunoprecipitation ETT in HEI-OC1 cells revealed enhanced tyrosine phosphorylation of ETT by E2 and T vs. non-treatment controls. This indicates that a phosphotyrosine switch may regulate ETT expression, supporting that tyrosine-related phosphorylation can be modulated by sex hormones via post-translational regulation mechanisms.

Conclusions: Ergothioneine (EGT) supplementation mitigates age-related hearing loss in female mice. EGT effectively crosses the blood-cochlear barrier and accumulates in key auditory structures, though its cochlear uptake is not accompanied by changes in ETT mRNA levels. Age-related declines in ETT protein expression occur in spiral ganglion neurons, and ETT phosphorylation is enhanced by estrogen and testosterone, suggesting that sex hormones modulate ETT through post-translational mechanisms rather than transcriptional regulation.

T3. RNA Expression Analysis in a Mouse Model With Hearing Loss Accelerated by Prenatal Stress

Satoshi Hara^{*1}, Kali Burke², Airi Sakyo¹, Firasat Ali Shah², Harumi Saeki¹, Tomoaki Ito¹, Hajime Orita¹, Kazusaku Kamiya¹, Yusuke Takata¹, Takashi Anzai¹, Fumihiko Matsumoto¹, Amanda Lauer², Kathy Gabrielson²

¹*Juntendo University*, ²*Johns Hopkins University*

Background: Prenatal stress resulting from pandemics, genocide, famines, and natural disasters has been linked in large population studies to adult-onset diseases. Recent studies have raised concerns about the disease risks in adult offspring exposed to prenatal stress during the global COVID-19 pandemic. In the auditory system, animal studies have reported audiological impacts of prenatal stress. To further investigate this relationship, we established a restraint-induced prenatal stress model in C57BL/6 mice, a strain known to develop progressive hearing loss in early to mid-adulthood. Using this model, we find that prenatal stress accelerates hearing loss as well as inner and outer hair cell loss. To elucidate the mechanisms underlying the association, we hypothesized that changes in gene expression associated with age-related hearing loss and oxidative stress play a key role in this process. To test this hypothesis, we analyzed cochlear RNA expression in the prenatal stress model of C57BL/6 mice.

Methods: Pregnant female C57BL/6 mice were assigned to either the prenatal stress group or the control group. The prenatal stress group was subjected to restraint stress in a pill tube for 2 hours per day over 5 consecutive days (gestation day 10.5-14.5) during pregnancy to expose the fetuses to prenatal stress. One male and one female offspring from each litter were assigned for analysis. (10 litters for audiological/pathological and 4 for gene expression analyses) Auditory brainstem responses (ABRs) to clicks and tone bursts (4–32 kHz, 10–90 dB SPL) were recorded from 4 to 24 weeks of age. Outer and inner hair cells were manually counted in cochlear whole mounts at 4 and 24 weeks. At 24 weeks, RNA extracted from the cochleae of offspring, and RT-qPCR was performed to assess the expression of the following genes: CDH23, MYO7A, SLC26A4, SLC7A8, GJB2, KCNQ4, CAT, SOD1, SOD2, NRF2, GPX3, MGST1, and NOS2.

Results: The ABR thresholds were not significantly different until 12 weeks of age. However, after 16 weeks, ABR thresholds at middle and high frequencies were significantly elevated in the prenatal stress group. Similarly, the incidence of hair cell loss was not significantly different at the age of 1 month, whereas at 6 months, outer and inner hair cell loss at high frequencies was significantly greater in the prenatal stress group. Among the tested genes, CDH23, MYO7A, and CAT showed significantly lower RNA expression in the prenatal stress group.

Conclusions: This study provides insights into the mechanisms underlying the audiological effects of prenatal stress. CDH23 and MYO7A, genes associated with age-related hearing loss that encode tip-link proteins connecting stereocilia on hair cells, together with CAT, which encodes the antioxidant enzyme catalase, that may play key roles in this process.

T4. Interrelationships Between Subcortical and Cortical Levels of Neural Processing

So Eun Park^{*1}

¹*East Tennessee State University*

Background: Brainstem abnormalities may alter successive stages of neural processing in the auditory pathway, resulting in larger cascading effects at the cortical level¹. Aging-related changes in auditory function may have an even stronger impact on these interactions^{2,3}. Still, it remains unclear how interactions between subcortical and cortical levels of processing are affected in the aging auditory system. Therefore, the study investigates potential interrelationships between lower and higher levels of auditory neural processing within the auditory pathway. It examines how aging and age-related hearing loss influence neuroelectric responses across different stages of auditory processing, comparing these patterns with those observed in healthy young adults with normal auditory function. The study also aims to determine whether amplitude-based and latency-based interrelationships reflect distinct neural mechanisms underlying auditory processing at subcortical and cortical levels of the auditory pathway.

Methods: The study included 30 young normal-hearing adults (YNH), 26 older adults with normal or near-normal hearing (ONH), and 26 older adults with age-related hearing loss (OHL). Auditory middle latency responses (AMLRs) and late latency responses (ALLRs) were recorded monaurally and ipsilaterally at 110 dB peSPL in response to the /ba/ syllable. Relationships between the Pa of the AMLR and N1–P2–N2 of the ALLR were examined for latency and amplitude using Pearson's correlation coefficients (r).

Results: Significant amplitude-based correlations were observed in the ONH and OHL groups, but not in the YNH group. In both older groups, enhanced Pa amplitude corresponded with increased N1, P2, and N2 amplitudes, independent of hearing loss, suggesting that lower-level processing may be functionally related to later cortical responses. Neural information may be overloaded at higher auditory stations through weakened filtering and increased central gain, reflecting age-related loss of inhibitory control. In contrast, YNH listeners showed no amplitude-based interrelationships, except for the correlation between Pa and N1 amplitudes. In the young healthy auditory system, higher cortical responses may be less dependent on early sensory encoding. Latency-based correlations were significant across all groups, with prolonged Pa latencies associated with delayed N1, P2, and N2 latencies regardless of age or hearing status. These findings suggest that subcortical timing delays may carry over to higher cortical responses and contribute to slower processing along the hierarchical auditory pathway.

Conclusions: The findings support functional interrelationships between subcortical and cortical auditory processing in the aging auditory system. Neuroelectric responses at lower levels of the auditory pathway may interact more strongly with higher cortical processing in older than in young healthy listeners. Amplitude and latency may reflect distinct neural mechanisms.

T5. Effects of Nerve Fiber Diameter and Myelination in Combined Stimulation in the Inner Ear Employing Realistic Human Inner Ear Models

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Background: The cochlea and vestibular organs, which sense hearing and spatial awareness, often suffer from sensory cell dysfunction or loss that requires electrical stimulation. Thus,

research explores stimulation through the combined use of cochlear and vestibular implants. In-silico models of the inner ear provide valuable input for these combined scenarios, as they offer the possibility to test multiple scenarios in advance of in vivo experiments and patient studies. Recently, we presented a modeling workflow for simulating the combined stimulation of the cochlea and the vestibular system. In this study, we employ this model to investigate the influence of fiber diameters, myelination, and disruption in the fiber models on the simulation output to mimic neural degeneration.

Methods: Based on a modeling workflow previously presented by our group, nerve fiber trajectories for the inner ear nerves were generated, including the cochlear and vestibular nerves. Those fibers were recognized mathematically for each nerve branch by two multi-compartment nerve models for the cochlear nerve and the vestibular nerve, respectively. Employing the finite element method, extracellular electrical fields resulting from electrode contact stimulation were computed. Various scenarios involving electrodes in both systems were simulated, allowing for detailed interpretation of possible position-specific differences. Crucial parts of the multi-compartment models include the diameters and the myelination of the fibers, both affecting the conduction velocity of the fibers. We varied both aspects in intervals realistic for human nerves, with parameters ranging from degenerated to healthy nerves, and estimated the effect of degeneration and different fiber distributions on the measured values in humans. Multiple electrode positions for measuring and stimulating electrodes in both organs were evaluated, relying on a realistic implant geometry.

Results: The ECAPs and AGFs are strongly dependent on the stimulating and measuring electrode. Furthermore, the fiber diameter distribution has a significant influence on the threshold of the measured fibers. The myelination of the fibers strongly affects the latency and the spike propagation in the inner ear nerves. This also results in increased AGF thresholds for lower myelination.

Conclusions: The presented model facilitates the exploration of the influence of combined cochlear-vestibular stimulation on both the cochlea and vestibular system. This will enhance our comprehension of how electrode placements and stimulation scenarios affect targeted stimulation within the inner ear, as well as the reciprocal influence when stimulating these organs. Moreover, this understanding could aid new diagnostic possibilities, as the model enables the investigation of distinctive impacts of pathological conditions on ECAPs, with a focus on neural degeneration.

T6. Social Frailty Predicts Three Month Hearing Aid Outcomes in Community Dwelling Older Adults

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Background: Older adults with hearing loss frequently exhibit reduced social engagement—termed social frailty—which has been linked to incident disability and poorer health outcomes as defined by Makizako et al. (2015). However, whether social frailty influences early benefit from hearing aids remains unclear. We aimed to determine the association between pre-fitting social frailty and 3-month hearing aid outcomes, while accounting for baseline audiologic performance and conversational exposure.

Methods: We conducted a prospective cohort study of adults ≥ 60 years with bilateral sensorineural hearing loss entering a hearing aid fitting program (May 2020–April 2025). Devices were fitted bilaterally with gain incrementally adjusted every 1–2 weeks. Social frailty was classified at baseline following Makizako et al. criteria. The primary outcome was the International Outcome Inventory for Hearing Aids (IOI-HA) mean item score at 3 months. Audiologic measures included unaided pure tone average (PTA; 0.5–3 kHz), unaided best word recognition score (WRS), best aided PTA and WRS. Weekly conversation time was recorded as a communicative exposure proxy. We fitted a prespecified multivariable linear regression with IOI-HA as the dependent variable and predictors: social frailty, conversation time, unaided PTA, and unaided best WRS. Exploratory interaction terms tested moderation by age and conversation time.

Results: Sixty-one participants were analyzed (median age 83 years [IQR 79–88]; 53% female); 50.8% met criteria for social frailty (31/61). Hearing aids improved hearing on average: Δ PTA +24.0 dB (IQR 15.0–30.0) and Δ WRS +7.5 points (IQR 0.0–20.0). Median IOI-HA was 3.9 [3.3–4.3].

In multivariable analysis, social frailty independently predicted lower IOI-HA at 3 months ($\beta = -0.27$, SE = 0.087; $p = 0.034$), as did greater unaided PTA ($\beta = -0.02$, SE = 0.0086; $p = 0.019$), whereas better unaided WRS predicted higher IOI-HA ($\beta = +0.01$, SE = 0.0045; $p = 0.011$). Conversation time was not significant ($p = 0.242$). Model fit: $N = 61$; $R^2 = 0.229$; adjusted $R^2 = 0.172$; $F(4,54) = 4.02$; $p = 0.006$. A significant frailty by age interaction was observed (Type III $p = 0.0025$), indicating that the adverse association of social frailty with IOI-HA was more pronounced at older ages. No interaction with conversation time was detected ($p = 0.839$). Sensitivity analyses yielded consistent inferences.

Conclusions: Pre-fitting social frailty is an independent, clinically meaningful predictor of reduced subjective benefit from hearing aids at 3 months, beyond baseline audibility and speech discrimination. The effect strengthens with advancing age, highlighting socially frail, oldest old adults as a priority group for augmented counseling and follow-up. These findings support routine screening for social frailty in hearing aid pathways and the development of adjunctive interventions (e.g., facilitated social participation or caregiver-involved training) to enhance outcomes. Future work should test targeted, social engagement-focused strategies in randomized or implementation studies and examine longer-term durability of benefit.

T7. The Ongoing Search for an Accelerated, Translational, and Functionally Relevant Mouse Model of Age-Related Hearing Loss

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Background: Age-related hearing loss (ARHL) is partially caused by lifelong exposure to noise, resulting in degeneration of hair cells, auditory nerve fibers, and the Stria Vascularis. Despite affecting millions of adults, ARHL currently has no FDA-approved treatments and screening therapeutic candidates for otoprotection is therefore essential. However, studying ARHL in wild type mice is time-intensive and expensive, requiring mice to age for 18–20 months before initial hearing loss is observed. Genetic models for accelerated ARHL are not generalizable to humans. Here, we developed a 99-day protocol that longitudinally exposes B6CBAF1/J mice to moderate

noise levels. While a single noise exposure at these levels is insufficient to induce permanent hearing loss, repeated, sub-traumatic exposures may result in accumulated damage that induces hearing loss earlier than would occur through natural aging. Hypothesizing similar pathogenesis between ARHL and our repeated noise-exposure model, we hope to use this model to screen drugs for protection against ARHL.

Methods: Male and female mice were included, as well as ovariectomized (OVX) mice to model post-menopausal females, as our lab has previously shown the protective effect of estrogen on hearing. Hearing ability was measured periodically via auditory brainstem response (ABR) and distortion product otoacoustic emissions (DPOAE). After auditory testing, cochleae, brains, and femurs were harvested for histological analyses. Bone health was assessed via micro-CT to study the effect of estrogen deprivation on bone mineral density. Our criterion for a “functionally relevant” hearing loss model is a 30–40 dB threshold shift at 32 kHz.

Results: Six 90 dB, 2-hour noise exposures once per week did not result in hearing loss in male, female, or OVX mice after 99 days. To accelerate the progression of hearing loss, we increased the intensity of our noise exposures to 95 dB and exposed our mice to noise twice per week. This resulted in animals suffering from mild high-frequency hearing loss as indicated by significant ABR threshold shifts. To increase the severity of high-frequency hearing loss and optimize our model for drug screening, we increased exposure intensity to 97 dB. This increased the threshold shifts our animals experienced, but not at the desired frequencies, prompting a reevaluation of our paradigm to induce gradual, adequate, and high frequency hearing loss. Additionally, OVX mice had significantly weaker bones just 3 months after the surgery than intact controls.

Conclusions: Our findings demonstrate the feasibility of developing a chronic noise-exposure model, indicative of ARHL, for otoprotective drug screening. However, our linear approach to increasing noise-exposure levels did not produce our desired result. We are testing a new paradigm with more frequent noise-exposures to produce gradual, high-frequency hearing loss. Using this approach, we aim to establish a rigorous and reproducible translational platform for identifying therapeutic compounds for ARHL.

T8. Noise Induced Hidden Hearing Loss is a High-Risk Factor to Influence Alzheimer’s Disease Development and Progression via Immune Dysreaction

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Background: Alzheimer’s disease (AD) is a common neurodegenerative disease. Its population has a rapid increase with a trend toward earlier onset in the last decades. However, the underlying mechanisms are unclear. It has been believed that environmental factors may play a major role in this increase. Our previous study demonstrated that noise as a common pollution factor in the modern world could be a high-risk factor to accelerate AD development and progression. In this study, we further explored the underlying mechanisms.

Methods: 5XFAD AD mice were used. Both 5XFAD and littermate wildtype (WT) mice at age of 3-4 months old were exposed to white noise (~98 dB SPL) for 2 hr and one time. ABR, DPOAE, and acoustic-evoked cortical potential (AECPP) were recorded to assess hearing function and brain activity. Acoustic startle response (ASR) has been used as behavioral test to assess dementia or cognition decline. The auditory cortex (AC), inferior colliculus (IC), cochlear

nucleus (CN), and cochleae were also collected for the bulk Poly(A) RNA Sequencing analyses to assess the genomic changes.

Results: After noise exposure to induce hidden hearing loss, the noise-exposed 5XFAD mice showed the fast decline in cognition measured by ASR. The decline-speed was 2-3 times faster than that in no-noise-exposed AD mice. RNA-Seq examination showed significant, different immune dysfunctions in the different auditory centers besides the significant downregulation of key synaptic and nerve signaling pathways. Further analysis of the interaction between genotype and noise exposure, i.e., “difference between difference”: (noise:5XFAD – control: 5XFAD) – (noise: WT – control: WT), different regions (i.e., AC, IC, CN, and cochlea) of 5XFAD mice demonstrated different reactions to noise. The AC showed exaggerated neuroinflammation, suggesting that AD not only caused baseline neuroinflammation but also appeared hyper-sensitive to noise stress. However, in the IC, noise crippled the immune system and shifted toward a desperate attempt to produce more energy and repair components, suggesting that noise caused the IC in the immuno-compromised state. Like the IC, the CN also showed a suppressed interferon response, but its compensatory mechanism appears to focus on structural changes. Finally, the cochlea showed a combined the metabolic stress observed in other auditory centers with a unique, non-interferon-based inflammatory response. This could lead to different types of synapse/tissue damage and hearing loss.

Conclusions: Noise can accelerate AD development and progression. AD mice have hyperinflammatory state after noise exposure. The difference between brain and cochlear further indicates that the interaction between AD and noise can trigger diverse, tissue-localized pathologies. This study also reveals that noise could induce widespread immune dysreaction throughout the brain and cochlea and provides important information for noise-induced hearing loss.

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T9. Vocalization and Sensory Gain Modulation in Primate Auditory Cortex

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Background: Vocal production is a sensory-motor process dependent upon auditory self-monitoring to detect and correct errors in vocal production. Previous work has described a robust vocalization-induced suppression of activity in auditory cortical neurons, while simultaneously increasing sensitivity to experimental perturbations in sensory feedback. These results have been suggested to represent vocal feedback error computation in the auditory cortex. However, the neural computations underlying these vocalization-related responses, and error calculation, remain unknown. Past studies have been limited by inherent variability in natural behaviors, like vocal production, which complicate quantitative comparison with measurements of passive sensory coding.

Methods: We investigated vocalization-related neural activity in the auditory cortex of marmoset monkeys, recording neural activity using implanted multi-electrode arrays in freely moving animals. We presented parametrically-varied tone stimuli during vocal production and compared responses to coding of identical sounds during passive listening in the laboratory

sound booth. To exclude effects of acoustic interaction, we also tested tones along with playback of vocal sounds.

Results: We found that responses to presented sounds were reduced during vocalization when compared to stimuli presented when an animal was not vocalizing, including control tones presented during vocal playback. Vocal production effects on sensory responses varied systematically across tone frequency for many neurons, suggestive of divisive gain modulation rather than a subtractive inhibition. There were no changes in frequency tuning attributable to vocal production. The relationship between individual tone frequencies and overlapping vocal acoustics, as well as receptive field properties, were also examined.

Conclusions: These results suggest gain modulation as a possible computational mechanisms underlying vocalization-induced suppression and vocal error calculation, and may suggest avenues for future of investigation of local cellular and circuit mechanisms involved in broader sensory prediction and sensory-motor processes.

T10. Developmental Trajectories of Auditory Thalamocortical Circuit

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Background: The auditory cortex is a key region for sound processing, bridging sound perception with higher-level cognitive, emotional, and multisensory processes. Among the ascending information flows, the thalamus is strategically positioned to relay acoustic and non-acoustic information to the cortex. The first-order pathway, comprising the ventral division of the medial geniculate body (MGBv), transmits acoustic information primarily to the primary auditory cortex (A1). However, the specific contributions MGBv thalamic afferents to early sensory processing, topographic organization, and the formation of tonotopic maps remain elusive.

Methods: To understand how thalamocortical afferents process information, it is essential first to determine when and where these thalamic inputs arrive in the auditory cortex. To distinguish MGBv afferents from those of higher-order thalamic nuclei and to preserve the thalamocortical tracts, we performed tissue clearing on brains from *Tshz1*tdTomato mice in which the MGBv axon projections are selectively labelled. Brain clearing renders the tissue optically transparent, allowing high-resolution, volumetric imaging of intact axonal projections with minimal photodamage and reduced imaging time. Next, to investigate how the development of MGBv afferents differs from the higher-order thalamic nuclei (i.e., dorsal division of MGB, MGBd), we crossed *Tshz1*-Flpo mice with *Calb*-Cre mice (Calretinin-expressing exclusively in MGBd), and injected the littermates' thalamus at birth with a combination of Flpo-dependent and Cre-dependent virus tagged with different fluorophores.

Results: We find that MGBv afferents innervate A1 at birth, with projections primarily ramifying in the deeper cortical layers. By postnatal day 3, MGBv afferents are visible throughout the cortex, and within two days, the processes in the deeper layers begin to prune, while those in the thalamorecipient layer (layers 3b/4) become denser. During the first postnatal week, MGBd afferents spanned a broad region. Still, they were notably absent from the layers 3b/4 of the areas innervated by the MGBv, suggesting that this non-overlapping region corresponds to A1, while the surrounding regions represent higher-order auditory cortex. With

age, the area innervated by MGBv afferents expanded along the anterior–posterior axis. Additionally, in nascent A1, the MGBd afferents were found to be sparsely innervating the area below the layers 3b/4. Notably, even in posterior nascent A1 regions where MGBv afferents are absent, MGBd afferents still ramify below layers 3b/4, suggesting that the A1 can be distinguished from surrounding higher-order auditory cortices as early as birth, although an earlier onset cannot be excluded.

Conclusions: Our findings demonstrate the differential developmental trajectories of auditory thalamocortical afferents in mice, and provide genetic tools for studying each thalamocortical pathway independently.

T11. Multimodal Profiling Reveals Cell-Type Diversity of Supragranular Pyramidal Neurons in the Macaque Auditory Cortex

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Background: The auditory cortex serves as a hub for higher-level processing of complex auditory signals. In vivo studies show that encoding of sound attributes by feature-selective neuronal ensembles is specialized across different areas of auditory cortex. It remains unclear if these distinctions are reflected in the biophysical properties of neurons, which can provide mechanistic insights into how signals are processed at different cortical levels. Given the expansion of cellular diversity in human temporal cortex and the complexity of auditory processing in primates, we sought to investigate cell-type specific regional specializations of excitatory neurons within the macaque auditory cortex. Here we present preliminary data profiling distinct neuron types in the macaque auditory cortex and their regional specializations.

Methods: To establish regional specializations at the cellular level, we performed patch-seq recordings from individual neurons in quiescent ex vivo slices pharmacologically isolated using synaptic blockers. Neocortical tissue was collected from primary auditory cortex (core regions) and auditory association cortex of M. Nemestrina (n = 8 female) and M. Mulatta (n = 2 male) macaques. Animal subjects ranged in age from 3-22 years old (mean age: 10.2 ± 2.1 years). The biophysical properties of neurons were collected using intracellular patch-clamp recordings from both acute and cultured ex vivo brain slices at a temperature of 30-32°C. At the end of each recording, the nucleus was extracted and processed for RNA sequencing. These data were used to establish transcriptomic identity in a subset of pyramidal neurons. Brain slices were then placed in fixative and histologically processed to reconstruct the morphological features of biocytin-filled cells.

Results: Data were collected from a total of 63 neurons (n = 19 caudal auditory core, n = 33 rostral auditory core, n = 11 associative auditory cortex). Electrophysiological recordings reveal that supragranular (layer 2/3) neurons located in the caudal areas of auditory core exhibit higher input resistance, higher firing rates, and action potentials with a shorter half-width compared to neurons in auditory association cortex. Supragranular neurons in the primary auditory cortex displayed hyperpolarization-activated cyclic nucleotide-gated (HCN)-dependent membrane properties. These properties include depolarized resting potentials, a prominent voltage sag, and enhanced subthreshold resonance that further increased with cortical depth.

Conclusions: As in other brain regions, macaque primary auditory cortex exhibits a defining feature that separates primates from rodents: the expression of several HCN mediated properties in layer 2/3 neurons. Additionally, across the macaque auditory cortex, layer 2/3 neurons can be distinguished based on several electrophysiological signatures. Neurons from the most caudal areas of the auditory core maintain faster firing properties than more rostral portions of auditory cortex. Collectively these findings suggest that different topographic regions of the primate auditory cortex are specialized at the level of individual neurons' intrinsic properties.

T12. Longitudinal Monitoring of Developmental Plasticity in the Mouse Auditory Cortex

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Background: The postnatal brain undergoes substantial plasticity to represent features and statistics of the sensory world. For the auditory system, studies in rodents (Zhang et al. 2001, Villers-Sidani et al. 2007, Dorn et al. 2010) and humans (Näätänen et al. 1997) indicate that the postnatal auditory cortex changes in response to early acoustic experience. While large-scale changes in responses to pure tones and representations of sound frequency have been described, technical challenges have limited our understanding of how these changes unfold over development across individual neurons and neuronal cell-types. Even less is known about how the developing auditory cortex processes spectrotemporally complex vocal sounds, such as ultrasonic vocalizations emitted by rodents in social contexts (Yao et al. 2023). How and when neuronal representations of auditory cues – from lower-dimensional features like spectral frequency to higher-dimensional auditory objects like vocalizations – first emerge in the postnatal auditory cortex and change with experience is unknown.

Methods: I performed longitudinal two-photon calcium imaging of hundreds of neurons in the auditory cortex of young mice from hearing onset into adulthood. The fluorescent calcium indicator GCaMP8m was expressed in excitatory (N=9 animals), inhibitory (N=4), or all neurons (N=7) for functionable imaging of distinct cell-types in cortical layers 2/3. Near-daily imaging from the same field of view of neurons commenced on postnatal day (P) 12 in response to pure tones (4-64 kHz at 70 dB SPL) and playbacks of pup ultrasonic vocalizations.

Results: Excitatory and inhibitory neurons in the auditory cortex started responding to pure tone stimuli by P13-14 with an initial tonotopic organization that expanded over the next few days. While a subset of neurons gradually changed their frequency tuning, the population-level representation of tone frequency remained largely stable across days, even at these early ages. In contrast, neurons in animals exposed to a repeated tone during the critical period for tonotopic plasticity (~P13-17) gradually shifted their tuning towards the experienced tone frequency. Inhibitory neurons already tuned to the exposed tone became transiently suppressed during the same period, suggesting that disinhibition may support experience-dependent remodeling of cell tuning during tonotopic map reorganization. Meanwhile, responses to ultrasonic stimuli – both pure tones and pup vocalization playback – emerged at later ages (> P16), were short-lived relative to lower-frequency sound responses, and were predicted by cells' tone frequency tuning at early, but not later, ages.

Conclusions: Neural representations of tone frequency emerge at hearing onset and remain largely stable under ‘typical’ developmental conditions, but are distinctly modified by early acoustic experience. In contrast, neuronal representations of vocalizations gradually change as they decouple from ultrasonic tuning through later development. Altogether, this work reveals cellular and circuit dynamics of auditory developmental plasticity that may underlie language acquisition and other essential aspects of auditory development.

T13. Deletion of the Transcription Factor Pou4f1 Alters the Epigenetic Landscape of Developing Spiral Ganglion Neurons

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Background: Pou4f1 is a transcription factor known to be involved in early spiral ganglion (SG) development. It is initially expressed broadly by immature spiral ganglion neurons (SGNs) but later becomes restricted to just the SGN IC subgroup. Germline knockout of Pou4f1 leads to a variety of defects in cochlear development including decreased SGN survival, altered positioning and axonal patterning, and premature hair cell development. Previous work has shown that Pou4f1 likely interacts with the transcription factor Isl1 to regulate the downstream genes controlling these developmental processes. However, differences in phenotypes between Pou4f1 and Isl1 mutants indicate Pou4f1 may also act on its own to regulate gene expression.

Methods: To assess changes in gene expression and chromatin accessibility in the absence of Pou4f1, wildtype and Pou4f1^{-/-} SGs were dissected at E14 and analyzed using either bulk RNA sequencing (RNASeq) or single-cell ATAC-seq (scATAC-seq). To assess common regulated genes Pou4f1^{-/-} RNASeq data was compared to existing published RNASeq data from E14 Isl1^{-/-} SGNs (GSE182575). Analysis of scATAC-Seq data was carried out using the R package Signac (V1.15).

Results: RNAseq data at E14 revealed 193 differentially expressed genes (DEGs) in the absence of Pou4f1 ($p \text{ adj} < 0.05$, $\text{Log}_2\text{FC} > 1.5$). Gene ontology analysis revealed these DEGs were enriched for roles in neuron development such as dendrite development, cell migration, neuron fate specification and innervation – all roles affected in the Pou4f1^{-/-} phenotype.

To assess whether Pou4f1 regulates these genes independently, or alongside Isl1, we compared the Pou4f1^{-/-} DEGs we identified at E14 with DEGs from a previously published Isl1^{-/-} RNA-seq study in the SGNs also at E14. We found that of the genes significantly up or down regulated in Pou4f1^{-/-} SGNs, only 32% were shared with Isl1^{-/-}. This indicates that over 2/3rds of genes regulated downstream of Pou4f1 do not also require the presence of Isl1.

scATAC-seq data at E14 revealed significant differences in chromatin accessibility in Pou4f1^{-/-} SGNs at E14, but not in adjacent cell types such as glia or mesenchyme. In addition, when directly comparing wildtype SGNs with Pou4f1^{-/-} SGNs, we found over 700 regions of differentially accessible chromatin. When these regions were mapped to their closest gene we found that 69% of DEGs identified in our RNA-seq data were associated with changes in chromatin accessibility in the absence of Pou4f1.

Conclusions: Overall these findings indicate that Pou4f1 is a key regulator of SGN development, and is able to independently regulate the expression of key developmental genes via broad changes to chromatin accessibility. Future work will focus on identifying direct binding sites of Pou4f1 using CUT and RUN, and subsequently identifying the networks of genes downstream of Pou4f1 which regulate specific developmental processes.

T14. Absence of the Ampa Receptor Auxiliary Subunit Tarp-Gamma 2 (Stargazin) Leads to Cochlear Synaptopathy and Progressive Hearing Loss in Female Mice

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Background: The pore-forming subunits of AMPA-type glutamate receptor/channels (AMPA-Rs) are complemented by auxiliary subunits which influence channel properties and mediate their membrane trafficking. Inter-synaptic heterogeneity of auxiliary subunits is thought to underlie the diversity of AMPAR functionalities observed in different synapses in different brain regions. The precise complement of pore-forming and auxiliary subunits expressed at a specific synapse is known in relatively few cell types. This is, to our knowledge, the first report on cochlear AMPAR auxiliary subunits.

Methods: TARP-gamma-2 KO mice on C57BL/6 background were crossed with CBA/CaJ to remove Cdh23 age-related hearing loss, and maintained on the mixed background. Genotypes were produced in the expected Mendelian ratios when crossing heterozygotes, but KO mice were ~25% smaller than littermates at 1 month. For in vivo auditory testing, mice were anesthetized with Avertin due to a lethal interaction of the KO with ketamine/xylazine. Hair cells and ribbon synapses were imaged and quantified with confocal microscopy. Synapse ultrastructure was assessed with TEM. Changes to the proteome were assessed with mass-spectrometry. Single-unit recordings in the auditory nerve and patch-clamp recordings on postsynaptic terminals in the cochlea are pending.

Results: ABR testing revealed a progressive threshold elevation from 2-6 months of age that was specific to female KO mice. DPOAEs were unaffected. Threshold elevations (~ 40 dB) plateaued after ~6 months and remained stable up to 2 years. At 1 year, female KO mice exhibited postsynaptic terminal swellings at the inner hair cell (IHC) ribbon synapses, characteristic of cochlear synaptopathy. Few synapses were missing per IHC, however, surprisingly there was significant loss of IHCs up to ~25% in the cochlear base. Outer hair cells appeared in tact. Immunolabelling of ribbon synapses revealed a reduction in the pore-forming subunit GluA2 in female KO mice. In older animals, both sexes had significant increases in proteins linked to complement pathway activation and mitochondrial organization. In younger animals, males displayed elevated levels of immune-associated proteins, whereas females showed reduced abundance of proteins involved in synaptogenesis and mitochondrial function.

Conclusions: The sex dependent progressive hearing loss in female TARP-gamma-2 KO mice is reminiscent of the phenotype of female GluA3 KO mice (Rutherford et al., 2023; Pal et al.,

2025). In both cases, synaptopathy was associated with terminal swellings and a specific loss of GluA2 subunits. The loss of GluA2 implies an increase in the abundance of calcium-permeable AMPARs at the ribbon synapses, although postsynaptic densities were smaller suggesting fewer overall AMPARs. Unlike the GluA3 KO mice, some IHCs were lost in aged TARP-gamma-2 KO mice but ribbon size was unaltered in surviving IHCs. These findings show that cochlear afferent synapses require TARP-gamma-2 for normal function and maintenance, although they initially form and underlie apparently normal ABR thresholds at 1 month of age.

T15. Differentiating Spontaneous Rate-Specific Responses of the Human Auditory Nerve via Forward Masking

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Background: Auditory nerve (AN) fibers transmit the output of cochlear hair cells to the brainstem. Fibers vary in their spontaneous rates (SR) of firing, with high SR fibers sensitive to lower-level sounds and vice versa. While SR-specific fiber responses can be directly recorded in animals, non-invasive measures in humans, i.e., auditory brainstem responses (ABRs) or the compound action potential (CAP) measured through electrocochleography, lack SR-specific resolution. Therefore, this study sought to propose a forward masking paradigm to better differentiate the electrophysiological response of AN fibers with different SRs in humans.

Methods: Twenty participants with normal hearing thresholds underwent the study. The stimuli consisted of five rapid click trains with one, two, three, four, or five clicks. The clicks in each train were spaced 1 ms apart, and each click was 15 dB higher in level than the one that preceded it (such that the clicks ranged from 45 to 105 dB peSPL in the train with five clicks). The preceding lower-level clicks within a train were presumed to serve as the forward maskers of the response to the next click, but only for fibers that were responsive to that lower level (and thus had higher spontaneous rates). Fiber-specific CAP responses to the final click of each train were isolated by subtracting the response to the train with one less click, canceling out neural responses to prior clicks.

Results: We found that preceding lower-level clicks in the train effectively masked the response of lower threshold AN fibers to subsequent clicks. Subtracting the responses to adjacent click trains allowed the activity from fibers that were responsive but not yet saturated to be isolated. At the highest level, some participants showed smaller SR-specific responses than the second-highest, indicating reduced response from low SR fibers.

Conclusions: Through our forward masking paradigm, we were able to isolate the responses from fibers with different spontaneous rates. Identifying fiber-specific response patterns not only helps to bridge the gap between human and animal research in auditory nerve function but also may also hold potential as a more sensitive proxy for peripheral neural degeneration, such as auditory neuropathy or cochlear synaptopathy.

T16. Spectrotemporal Modulation Coding in the Auditory Nerve: Mechanisms and Implications for Hearing Loss

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Background: Spectrotemporal modulation (STM) sensitivity is a strong behavioral predictor of speech-in-noise performance, likely because STM patterns are present in natural speech. However, the neural mechanisms behind this predictive power remain unclear. A key question is which cues listeners rely on when detecting STM, such as envelope, temporal fine structure (TFS), or dynamic place coding, and how these cues are affected by sensorineural hearing loss (SNHL). We hypothesize that SNHL can affect auditory peripheral coding in response to STM through several mechanisms, including reduced frequency selectivity, degraded phase-locking precision, cochlear deafferentation, and distorted cochlear tonotopy (DT). In DT, tuning curves from auditory nerve (AN) fibers with SNHL often show a hypersensitive low-frequency tail and a reduced tip-to-tail ratio, resulting in off-frequency responses that alter the normal frequency-to-place mapping. To test this hypothesis and characterize how STM is encoded in the auditory periphery under normal and impaired conditions, we record from single AN fibers in chinchilla models of normal hearing and SNHL in response to STM stimuli. In parallel, we use a computational AN model to explore stimulus parameters and validate analysis methods.

Methods: We record from single-unit AN fibers in response to STM stimuli in chinchilla, alongside simulated responses from a computational AN model. Spike-train analyses assessed (1) envelope coding via phase-locking to modulation envelopes, (2) TFS coding using interspike interval analyses, and (3) dynamic place coding using moving-window averages across characteristic frequencies (CFs). To overcome sparse CF sampling in physiology, we employed the Spectrotemporal Manipulation Procedure (STMP; Heinz 2007; 2010), which predicts population responses from single AN fibers by varying the stimulus sampling rate. This reduces variability across fibers and enables predictions across a range of CFs.

Results: Preliminary analyses of normal-hearing chinchilla AN single-unit recordings and corresponding model simulations suggest that STM is encoded through complementary envelope, TFS, and place cues. Early results also show that STMP is feasible in both model and physiology, providing a robust method to estimate population responses from limited single-unit recordings. Modeling further allows us to explore a wider STM parameter space than is possible experimentally, supporting optimized stimulus design for cross-species studies.

Conclusions: Our preliminary results provide a foundation for understanding how spectrotemporal modulation (STM) cues are represented in the normal-hearing auditory nerve. Prior work suggests that sensorineural hearing loss (SNHL) may degrade STM encoding through mechanisms such as reduced frequency selectivity, degraded phase locking, and distorted tonotopy. We will extend our approach to preclinical chinchilla models with distinct pathologies to directly test how different forms of hearing loss alter STM coding. This work will help clarify the mechanisms underlying STM sensitivity and how they are disrupted by hearing loss, providing a stronger mechanistic basis for translational predictions.

T17. Contralateral Sound Attenuation Can Improve Speech-In-Noise Intelligibility for Bilateral Cochlear Implant Users

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Background: We have shown elsewhere that contralateral sound attenuation (CSA) can improve speech intelligibility for normal-hearing listeners (Lopez-Poveda et al., 2022, *Hear Res* 409:108469) as well as hearing-aid users (San-Victoriano et al., 2023, *Hear Res* 432:108743) in simulated free-field, “cocktail party” listening situations. The aim here is to evaluate whether CSA may also benefit users of bilateral cochlear implants.

Methods: CSA was implemented at the front-end of processing and in combination with a pair of functionally independent MED-EL FS4 audio processors, one per ear. CI user performance with and without CSA was assessed by comparing (1) speech reception thresholds for sentences in fluctuating and steady-state noise, in unilateral and bilateral listening modes, and for three different speech levels; and (2) sound source localization in quiet and in noise. Five users of bilateral CI participated in the tests.

Results: CSA improved speech intelligibility in both fluctuating and stationary maskers across all the spatial configurations tested, except for target and noise sources co-located in front of the listener, where CSA did not affect intelligibility. Also, CSA slightly improved sound source localization.

Conclusions: CSA can be implemented at the front-end of processing. It is simple and effective, which makes it potentially suitable for implementation in CIs and any pair of auditory prostheses. (Work supported by MED-EL GmbH).

T18. Assessing the Safety and Efficacy of Ultrasound as a Novel Hearing Aid Device

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Background: Although ultrasound (US, > 20kHz) is above conventional ranges of human hearing, previous studies in guinea pigs have demonstrated that US can activate the ascending auditory pathway. Activation was abolished by the removal of cochlear fluid, supporting that the response originates from the cochlea rather than direct excitation of neurons. Additionally, studies have shown that amplitude-modulated US with modulation frequencies in audible ranges can undergo self-demodulation through non-linear propagation, making the US perceivable to humans. This study systematically investigates the response properties of neurons in the inferior colliculus (IC) to modulated US compared to pure tones and evaluates a safe range of US parameters to activate the auditory system.

Methods: In acute guinea pig experiments, multiunit IC activity was recorded using a two-shank 32-channel electrode array. Stimuli included broadband noise, pure tones (1-40 kHz, 0-70 dB SPL), unmodulated US (430 kHz carrier, 50 ms pulse, 5 ms ramps, 3-117 kPa), and modulated US (1-40 kHz, 3-63 kPa.) Acoustic tones were delivered via closed-field speakers and US was applied using a 430 kHz transducer coupled to the dura. In chronic experiments, animals received 10 minutes of continuous transcranial US (e.g. 100 kPa, 50 ms, 430 kHz unmodulated, 5 ms onset-offset ramps, 50% duty cycle). Distortion product otoacoustic emissions (DPOAE), as well as broadband and pure tone auditory brainstem responses (ABRs) were measured pre-exposure, post-exposure, day 7 and day 14.

Results: US stimulation showed similar frequency tuning to acoustic stimulation. Thresholds were between 20-40 kPa, with the lowest US thresholds for modulation frequencies around 8kHz, consistent with pure tone stimulation. Firing rates increased monotonically with US pressure reaching a plateau. The average dynamic range was 26 kPa (equivalent to 6 dB re: 1 kPa increase). There were some differences in IC responses spectrally and temporally between US and acoustic stimulation. Frequency tuning remained relatively narrow for US stimulation with increasing pressure, and some channels showed a secondary peak at subharmonic modulation frequencies. Unmodulated US produced broad IC activation with primarily onset-suppression patterns, compared to onset-sustained responses typical of the central IC. In chronic experiments, 10-minute US exposure at 50 kPa did not produce threshold shifts in ABR or DPOAE beyond normal inter-animal variability observed in hearing control animals.

Conclusions: Our results confirm that ultrasound can activate the auditory system and appears to be safe with stable hearing thresholds. US stimulation shows potential as a novel hearing technology by bypassing the air-conduction pathway and could overcome limitations of conventional hearing aids. Further studies are needed to determine how varying stimulation patterns with US affect neural coding of complex sounds like speech and music.

T19. How Loud is a Vibrating Ci Electrode

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Background: Patients with severe sensorineural hearing loss can benefit from cochlear implants (CI), which stimulate the auditory nerve and bypass damage to the outer hair cells. In patients with residual hearing, a hybrid solution combining CI and hearing aids supports auditory rehabilitation. However, in some cases—such as patients with more pronounced mixed hearing loss—hearing aids are insufficient. For these type of patients, an active vibrational stimulation approach, similar to active middle ear implants (AMEI), in addition to a CI, may represent a viable solution. In this study, we investigated the stimulation output that can be achieved by a vibrating CI electrode in human cadaver temporal bones (TBs), using intracochlear pressure measurements.

Methods: A posterior tympanotomy provided wide access to the round window in 10 temporal bone specimens. Stapes displacement in response to sound pressure levels (SPLs) in the external auditory canal was measured and compared to ASTM standards. Acoustic stimulation was generated using a loudspeaker (DT48, Beyerdynamic, Germany) attached to an ear funnel and cemented into the ear canal. A probe microphone (ER7C, Etymotic Research, Inc., USA) measured sound pressure near the tympanic membrane (< 2 mm). An optical pressure sensor (FISO, Quebec, Canada) was inserted into the scala vestibuli through a cochleostomy and fixed at the promontory. A CI electrode (Flex 20, MED-EL, Austria) was inserted into the scala tympani via the round window and coupled to a piezoelectric transducer to provide vibrational stimulation. Intracochlear pressure in the scala vestibuli (PSV) was recorded in response to both acoustic stimulation in the ear canal and to electromechanical CI vibration.

Results: PSV magnitudes relative to ear canal SPLs peaked at ~1 kHz in all TBs for acoustic stimulation in the ear canal. The vibrating CI electrode at 1 μm displacement generated equivalent SPLs of 107 dB between 0.1 and 2 kHz. At frequencies above 2 kHz, lower displacement levels were sufficient to achieve above 120 equivalent dB SPL.

Conclusions: These findings indicate that vibration of the CI electrode can provide sufficient output levels to support patients with mixed hearing loss (MHL) who retain residual hearing.

T20. Real-Time Brain-Controlled Selective Hearing Enhances Speech Perception in Multi-Talker Environments

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Background: Environments with multiple competing talkers and background noise create significant challenges for focusing on a single talker, particularly for individuals with hearing difficulties. Current hearing aids rely on techniques like directional microphones and beamforming, which often fail to align with the wearer's dynamic listening intent. Auditory attention decoding (AAD) presents a promising alternative by using listener's neural data to identify their target of attention and selectively enhance the attended talker. While previous studies have demonstrated decoding feasibility, it has remained unknown whether this approach can deliver real-time perceptual benefits.

Methods: We developed and evaluated a real-time closed-loop AAD system with four self-reported normal-hearing participants (S1-S4) implanted with brain electrodes during epilepsy treatment. Participants were tasked with focusing on one of two competing conversations while the system decoded their listening intent in real time and enhance the attended conversation. The ability of the system to handle instructed and self-initiated attention shifts was also tested.

Results: The system accurately decoded and enhanced the attended conversation. This led to significant improvements in speech intelligibility and reduced listening effort, as corroborated by pupillometry. Importantly, participants could seamlessly switch attention between the two competing conversations, with the system adapting dynamically to their shifts in focus, for both instructed and self-initiated attention shifts. All participants reported that the system would be useful, and psychophysical testing on individuals with hearing impairments further validated its effectiveness.

Conclusions: By providing the first direct evidence that a real-time, brain-controlled hearing system can improve speech perception, this work establishes a critical performance and viability benchmark for future auditory brain-computer interfaces (BCIs) and advances AAD from a theoretical concept to a validated solution for personalized assistive and augmented hearing.

T21. Effect of Reduced Stimulation Rates on the Perception of Phonemes, Sentences, and Pitch by Cochlear-Implant Users

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Background: Contemporary cochlear implant (CI) processing strategies use high pulse rates. The Temporal Integrator Processing Strategy (TIPS) removes pulses that are likely to be masked, substantially reducing expected power consumption while producing improved or equivalent speech perception (Lamping et al., 2020; Shahidi et al., 2025). However, it must be determined whether the potential power savings conferred by TIPS could be more simply achieved by reducing the channel stimulation rate without compromising speech perception. Some studies of speech perception at low channel stimulation rates suggest that speech intelligibility can be maintained with rates below the participants' clinical stimulation rate (Friesen et al. 2005; Weber et al. 2007; Brochier et al. 2017), with one study indicating that stimulation rates could be reduced to 50 pulses per second (pps) before phoneme recognition was significantly impaired (Fu and Shannon, 2002). Here we study speech perception as a function of stimulation rate using complementary measures of phoneme, sentence, and pitch perception.

Methods: A double-blind within-subject experiment tested twelve recipients of Cochlear™ devices across seven channel stimulation rates: 80, 110, 160, 240, 350, 500, and 900 pps. Speech perception was measured as consonant recognition for vowel-consonant-vowel pseudo-words; vowel recognition for consonant-vowel-consonant words; speech intelligibility of open-set sentences as the percent of words recognised in quiet or the speech reception threshold in multi-talker babble noise; and pitch ranking of 1/3 octave shifts in the fundamental frequency of a word. Phoneme discrimination performance was quantified using percent correct and mutual information measures and an information transmission analysis of articulatory features. Prior to testing each subject, threshold and most-comfortable levels were set for each rate and loudness was balanced across rates. Measures were taken acutely with no acclimatisation.

Results: There was a significant effect of rate for vowels, consonants, and sentences in quiet and noise, with data collection ongoing for the pitch-ranking task. Subjects demonstrated idiosyncratic patterns of phoneme recognition errors and performance across rates, with average percent-correct scores gradually declining with decreasing rate. Rate most impacted the transmission of consonant articulatory features of voicing, with a lesser effect on features of manner and place. Rate did not impact the transmission of vowel articulatory features. For sentences in quiet and noise and pitch-ranking results to date, performance typically worsened at rates below 160-240 pps. Pairwise comparisons performed on the complete dataset will indicate which rates result in significantly worse speech outcomes.

Conclusions: Speech perception is robust to reductions in channel stimulation rate well below those used clinically. Channel stimulation rates as low as 160 pps appear to accurately convey speech information, with sentence intelligibility and pitch ranking being more sensitive to the effect of rate than phoneme perception. A follow-up experiment will compare speech information transmission with TIPS and low stimulation rates.

T22. Three-Dimensional Analysis of the Effects of Chronic Implantation and Stimulation on Hearing

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Background: Chronic cochlear implantation and stimulation is known to produce a fibrotic tissue response within the cochlea. This fibrosis would be expected to impact residual hearing; however, previous studies have failed to produce a conclusive correlation between the two. We hypothesise this lack of correlation may be due to limitations in conventional histological techniques – analysis from two-dimensional microtome sections, being unrepresentative of the complex response that occurs following chronic implantation and stimulation. We have leveraged recent developments in tissue clearing – a process whereby normally opaque tissue is rendered transparent - and developed a method to allow for three-dimensional characterisation of the cochlear structures and fibrotic response to chronic implantation and stimulation within an intact cochlea.

Methods: Normal hearing guinea pigs (n=4) were bilaterally noise deafened (10kHz 120dB SPL free-field for 1 hour). After 14 days the left cochlea was implanted with an 8-contact intracochlear electrode array and received stimulation (50 μ C/cm²) for 4 weeks from a clinical stimulator and speech processor. Right cochlea served as deafened, unimplanted controls. Hearing assessments (acoustically evoked compound action potentials, aCAPs, 8 – 32kHz) were performed at implantation and at the conclusion of the chronic stimulation period. Cochleae were collected (with implant left in-situ); immunofluorescently stained with markers for cochlear structures (hair cells and spiral ganglion neurons, SGN) and fibrosis (e.g. fibroblasts and macrophages); and cleared in ethyl cinnamate. The whole cochlea was imaged on a light-sheet fluorescence microscope, and images volumetrically rendered and analysed using Imaris software.

Results: Noise deafening resulted in a significant increase of 58dB (SEM=2.3) at 8-32kHz (n=8 ears). In two animals, chronic implantation and stimulation resulted in a 1.5dB increase in hearing thresholds at 1-32kHz. Histological analysis is ongoing; preliminary analysis indicates chronic implantation and stimulation resulted in fibrotic growth, although this did not appear to affect hair cell or SGN survival.

Conclusions: Three-dimensional visualisation of the cochlea offers unprecedented insight into how the fibrotic tissue response to chronic implantation and stimulation can affect hair cell and spiral ganglion neuron survival. In addition, imaging the cochlea with the implant left in-situ allowed for the identification of the fibrosis and cochlear structures in relation to specific electrodes. Using a range of pathophysiological markers, this study demonstrates a novel method that provides an accurate assessment of the cochlea following chronic implantation and stimulation. This improved validation tool will be used in developing treatments to reverse or prevent cochlea damage.

T23. Comparative Spatiotemporal Dynamics of Four-Point Impedance, Access Resistance, and Polarization Impedance in Standard and Dexamethasone-Eluting Electrode Arrays

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Background: Intracochlear injury during surgical placement of a cochlear implant (CI) electrode array (EA) and the immune response to a foreign body can result in fibrotic scar formation and neo-ossification. Scar formation decreases the efficiency of electrical stimulation and can inhibit

residual acoustic processing. Electrode impedance is sensitive to intracochlear fibrosis and increases over time post implantation. Our center is evaluating subjects who have received dexamethasone-eluting electrode arrays (Dex-EA, which are intended to reduce the foreign body response to the CI. This study aims to evaluate electrode impedances over time within recipients of standard EA and Dex-EA. We hypothesize that impedance measures will be lower and more stable over time in Dex-EA implanted ears compared to controls.

Methods: 18 subjects were implanted with a standard Cochlear 632 array while 19 subjects were implanted with an experimental Cochlear 632D array. Electrode impedance measurements were performed at initial activation (typically 2 weeks after surgery) and at subsequent visits after initial activation at 2 weeks, 1 month, 3 months, 6 months, and 12 months. Three types of impedance measures were analyzed: access resistance, polarization impedance, and 4-point impedance. Access resistance and polarization impedance were derived from transimpedance matrix measurements obtained in a monopolar configuration, in which the stimulating electrode was intracochlear while the reference electrode was extracochlear. In contrast, 4-point impedance measurements were obtained in a bipolar configuration, in which both the stimulating and reference electrodes were intracochlear.

Results: In control subjects, access resistance increased over time, starting from the most basal electrodes and progressing apically with time. Impedances were lower for individuals with Dex-EAs across all electrodes at all observed time points. Polarization impedance in controls decreased after IA with more stability between 3 and 12 months. In comparison, impedances were lower for individuals with Dex-EAs across all electrodes at all observed time points. Four-point impedance in controls increased over time, starting from the most basal electrodes and progressing apically with time. In comparison, impedances were lower and more stable for individuals with Dex-EAs across all electrodes at all observed time points. Between 6 and 12 months, 4-point impedance demonstrated greater sensitivity to ongoing impedance changes compared with access resistance and polarization impedance.

Conclusions: Dexamethasone elution appears a promising strategy to reduce electrode impedance following CI surgery. 4-point impedance is more sensitive at detecting ongoing impedances changes between 6-12 months as compared to access resistance and polarization impedance.

T24. The Acoustic Change Complex in Response to Frequency Changes Along the Cochlear Implant Electrode Array

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Background: The Acoustic Change Complex (ACC), an auditory potential triggered by a change in an ongoing stimulus, has shown promising results as an objective measure for frequency discrimination and speech perception (Vonck et al., 2022). Although a wide variety of frequency change stimuli have been employed in previous studies, limited research has

specifically investigated the ACC for both low and high frequency tones in cochlear implant (CI) users with full electrode coverage. Addressing this gap, the present study investigates the ACC in response to frequency changes along the CI electrode array. We aim to enhance our understanding of frequency-specific auditory processing in CI-users and evaluate the clinical utility of the ACC as a marker for speech perception in this population.

Methods: This cross-sectional study utilizes a four-electrode EEG setup, with active electrodes positioned at Cz and Fz. Auditory stimuli consist of a 3000 ms pure tone base frequency, followed by an upward or downward frequency-modulated sweep, ending with a 500 ms target tone that is either 50% higher or lower in frequency (similar to Van Heteren et al., 2022). The lowest used frequency is 125 Hz, and the highest frequency is 6000 Hz. Stimuli are delivered directly to the CI via an audio input cable. In addition, participants complete speech perception tests in both quiet and noise, as well as spectral ripple discrimination tasks. Patient recruitment is ongoing, with 16 participants tested to date. By the time of the conference, a larger sample size will be reached and analysed.

Results: Preliminary data indicate that a distinct N1P2 waveform can be reliably elicited across all frequency change stimuli along the electrode array. In addition, N1P2 amplitudes are significantly smaller when the frequency transitions occur toward the ends of the array. Specifically, transitions from 188 Hz to 125 Hz produce smaller amplitudes compared to transitions from 125 Hz to 188 Hz, and similarly, transitions from 4000 Hz to 6000 Hz yield smaller responses than those from 6000 Hz to 4000 Hz. We observed moderate to strong positive correlations between hearing performance assessed with spectral ripple discrimination and speech perception, and N1P2 amplitudes for ACC in the mid-frequency range (500-750Hz, 2000- 3000Hz).

Conclusions: By the time of the conference, a larger sample size will be reached and analysed. The effects of frequency change stimuli along the electrode array, as well as the impact of upward and downward frequency changes, will be discussed. In addition, the relation between the different frequency change stimuli and speech perception, including speech in quiet, speech in noise, and spectral ripple discrimination, will be discussed.

T25. Modeling the Number of Neurons Activated at ‘Most Comfortable Level’ in Cochlear Implant Users

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Background: Cochlear implants are highly successful neural prosthetics, but the outcomes vary widely between users. A likely cause of this variability relates to the electrode-neuron interface. Prior modeling efforts have investigated factors of the electrode-neuron interface including 1) the density of neurons near each electrode and 2) the distance between each electrode and nearby neurons. These efforts have mainly focused on outcomes resulting from research-related measures (i.e., monopolar and tripolar thresholds). However, to make modeling efforts more clinically applicable, the model must be tailored towards measures used clinically (i.e., ‘most comfortable levels’). Thus, the purpose of this study was to build upon prior modeling structures of the electrode-neuron interface to incorporate most comfortable levels.

Methods: This study utilized a simplified geometry model of the cochlea, described in detail in Perkel et al. (2025). To tailor the model to apply to clinical data, parameters related to neural activation were optimized. Once the model was used to infer electrode position and fractional neuronal density from monopolar and tripolar thresholds, the number of activated neurons at the CI user's perceptual 'most comfortable level' was calculated. This process was applied to the monopolar 'most comfortable level' data for each channel in 18 adult cochlear implant users.

Results: We successfully optimized the model to be able to apply the 'most comfortable levels' by adjusting parameters related to the slope and center of the neuronal activation function. These parameters were adjusted to account for the intuitive increase in excitation spread from threshold levels (activating 100 neurons) to stimulating at 'most comfortable level'. On average, 502 neurons were activated (SD = 352, range = 48-2493), about five times the number of neurons arbitrarily used in prior models for detection threshold stimuli.

Conclusions: This novel finding provides a greater understanding of the electrode-neuron interface by relating estimated active neurons to behavioral thresholds and 'most-comfortable levels'. As this is the first study to model the number of neurons active with clinical data, it is interesting to note the large inter-patient variability. Importantly, because this level of neuronal information is impossible to measure in living humans, future studies will validate this model using temporal bones donated by cochlear implant users.

T26. Decoding Selective Attention in Cochlear Implant Users With Ipsilateral Residual Hearing

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Background: Cochlear implant (CI) users with ipsilateral residual hearing combine acoustic stimulation (AS) with electric stimulation (ES). Combined electric acoustic stimulation (EAS) usually improves speech perception compared to using ES or AS alone, most probably due to integration of both stimulation modalities. However, existing measures to assess EAS benefit rely primarily on behavioral tests, which provide limited insights into the neural mechanisms underlying electric acoustic integration. Objective measures based on EEG may offer a deeper understanding of electric acoustic integration at neural level. Furthermore, developed objective measures could be applied to populations where behavioral testing is challenging, such as young children or individuals unable to provide reliable responses.

Methods: Seven EAS users participated in the study. First, behavioral speech understanding was assessed in three listening modes: ES, AS, and EAS. Afterwards, selective attention was measured using EEG. During the selective attention task, two concurrent speech streams were presented and participants were asked to follow the assigned speech stream when listening with ES, AS and EAS. Both behavioral and objective measures were analyzed in terms of EAS benefit, which was defined as the difference in performance between EAS and AS. Moreover, integration efficiency (IE) was explored and calculated as a ratio between predicted and measured EAS performances for behavioral and objective measures. Finally, the correlation between EAS benefit and the amount of residual acoustic hearing as well as between integration efficiency and residual hearing were examined.

Results: Behavioral speech understanding significantly improved with EAS compared to both ES and AS alone. No significant correlation was observed between behavioral EAS benefit and the amount of residual hearing. However, a significant linear correlation between IE calculated for the behavioral speech score and amount of residual hearing was observed. EEG based selective attention decoding revealed a significant distinction between attended and unattended speech only under the EAS condition. Furthermore, selective attention EAS benefit showed a significant correlation with the amount of residual acoustic hearing. No correlation between selective attention IE and residual hearing was found.

Conclusions: Listening with EAS significantly improves behavioral speech understanding compared to ES and AS. Moreover, the speech understanding IE significantly correlated with amount of residual hearing. EEG based selective attention decoding demonstrated that the use of combined modalities enhances neural processing supporting selective attention. Additionally, the correlation between selective attention EAS benefit and residual hearing highlights the sensitivity of EEG based neural measures for assessing EAS benefit, demonstrating the potential of selective attention decoding as an objective measure to assess electric acoustic integration.

T27. End-To-End Deep Sound Coding Strategies for Enhanced Speech-In-Noise Understanding in Cochlear Implant Users

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Background: Cochlear implant (CI) users often face significant challenges in understanding speech in noisy environments. We present an overview of our work on novel deep learning-based sound coding strategies designed to improve speech intelligibility for CI users. These strategies span from front-end source separation algorithms to a fully end-to-end sound coding framework that directly estimates electrograms from raw audio input, bypassing traditional CI processing pipelines.

Methods: Our end-to-end approach, referred to as DeepACE, DeepF120, or DeepFS4, depending on the CI coding strategy it replaces, leverages deep neural networks (DNNs) to substitute conventional sound coding algorithms. Note that the “classical” sound coding strategies are needed during the DNN training. Building on prior work, we introduce several architectural innovations. We incorporate a discriminator network to enforce the generation of high-fidelity electrograms through adversarial learning, enhancing the effectiveness of the electrical stimulation patterns. Furthermore, we extend our framework to bilateral CI processing by introducing latent fusion layers that connect two monaural deep denoising sound coding networks. These layers multiply the latent representations from each side, facilitating interaural information sharing and improving generalization and noise reduction capabilities. We further provide insights into extending end-to-end sound coding strategies for brain-informed speech enhancement by integrating fusion layers that decode electroencephalography (EEG)-derived speech envelopes. These neural signals guide the DNNs toward the attended speaker. Finally, we demonstrate the feasibility of deploying these architectures on low-power, low-latency hardware platforms, supporting real-time implementation in next-generation CIs.

We evaluated the proposed strategies using both objective instrumental metrics and behavioral experiments with CI users. Comparisons were made against baseline methods including the classic Wiener filter and the state-of-the-art TasNet architecture used as a front-end to ACE, FS4, and F120. Behavioral testing involved the HSM sentence test in quiet and in ICRA7 noise conditions.

Results: Results from objective measures demonstrate that the end-to-end sound coding strategies significantly outperform baseline methods in terms of predicted speech intelligibility and noise reduction. The inclusion of the generative module and fusion layers further enhances performance. Speech understanding results in three studies including Nucleus, Advanced Bionics and MED-EL CI users show that performance in quiet was comparable across end-to-end sound coding strategies, and classical sound coding strategies with and without front-end processing. Notably, in noisy conditions, bilateral CI users achieved speech intelligibility scores with the end-to-end denoising sound coding strategy that approached those obtained in quiet, highlighting the potential of these methods to improve real-world listening experiences. Our results also show that incorporating EEG-based attention improves speech enhancement.

Conclusions: These findings suggest that DNN-based end-to-end sound coding strategies, also with extension to bilateral processing, generative and EEG-based brain informed components, offer a promising avenue for improving speech-in-noise understanding in CI users.

T28. Optical Detection of Basilar Membrane Damage

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Background: Hearing impairment affects over 1.5 billion people worldwide. More than 430 million people experience disabling hearing loss that demands rehabilitation. For patients with severe-to-profound hearing loss, cochlear implants (CIs) can restore some auditory perception.

Despite their success, CI surgeries pose challenges. Inserting the electrode array can misalign or damage the cochlea, causing inflammation and additional hearing loss in up to 40% of implantations. Existing monitoring techniques rely mainly on the surgeon's tactile feedback, with no direct real-time visualization. This increases the risk of structural damage, underscoring the need for better preservation methods to maintain residual hearing and improve CI outcomes. Advances, including robotic insertion, optical coherence tomography, and micro-cameras, show promise. Nevertheless, there is still no clinical tool to visualize and continuously assess the cochlea's structural integrity during electrode insertion.

Optical-based label-free methods, such as birefringence, allow the assessment of tissue organization without the need for staining. Birefringence arises from the interaction of polarized light with anisotropic tissues, where differences in refractive indices create a slow and fast axis within the structures. In the cochlea, the basilar membrane shows birefringence due to the radial arrangement of collagen fibers, which extend from the osseous spiral lamina to the spiral ligament, perpendicular to the cochlear lumen. These fibers contribute to the mechanical properties of the membrane, which are essential for the cochlea's tonotopic frequency mapping.

In this study, we aim to evaluate whether birefringence is a sensitive and reliable marker for tissue damage, with a particular focus on the cochlea. To investigate this, we performed both

qualitative and quantitative birefringence assessments on various tissues, including cornea, connective tissue, and cochlea from a mouse, all exposed to laser-induced damage. These measurements explore the potential of birefringence for monitoring tissue health and its use in real-time evaluation during CI electrode insertion.

Methods: In our study, we performed qualitative and quantitative birefringence measurements on unstained cornea, connective tissue, and cochlea sections from mice, males and females, each with a thickness of 10-20 micrometres. We obtained qualitative birefringence data using a conventional transmission-mode birefringence microscope, followed by quantitative analysis with an LC-PolScope microscope. We analysed normal cochlear samples and those exposed to various damage conditions, including laser light exposure (Omniguide CO2 laser).

Results: Orientation of collagen fibers was imaged in cochlear cross sections of normal mice with high sensitivity and resolution. Following cochlear damage, the retardation was close to zero, and the collagen fibers were no longer seen.

Conclusions: Other research groups have previously employed this method to evaluate the resolution of normal cochlear sections. In this context, we have shown its implementation in a clinical scenario involving cochlear damage. Further improvements will focus on evaluating these quantitative measurements in back-reflection mode.

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T29. The Opto-Electrical Cochlear Implant

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Background: Cochlear implants (CIs) successfully restore some hearing in severely-profoundly deaf. Contemporary CIs rely on electrical stimulation, with low spatial neural stimulation originating from the current spread, which limits the number of independent frequency bands or channels that can be processed simultaneously. It has been argued that more independent channels result in a better performance of CI users. Neural stimulation with light offers a promising alternative, enabling more selective targeting of neuron populations.

This work presents design considerations, development, and prototype testing of a novel fully implantable hybrid cochlear implant. The implant integrates electrical stimulation with optical components to explore light-based neural activation.

Methods: The oeCI prototype has 42 stimulation channels: 24 electrical (expandable to 32) and 18 optical. The system comprises four main parts: A) an electrical driver with 24 current stimulators, charge-recovery circuitry; B) an optical driver with 18 channels and adjustable switching rates; C) an ultra-low power processor; and D) a MEMS microphone. With small dimensions of 3.5×3.5×0.8cm³, the oeCI is designed to operate in three modes: electrical-only, optical-only, or hybrid. This configuration can be adjusted based on spatial selectivity and power requirements. The coding strategy encodes acoustic input by calculating spike probabilities based on signal energy or amplitude, with optional amplitude modulation. Phase information is incorporated by aligning pulse probability peaks with integer multiples of the center frequency,

preserving temporal fine structure. Envelope cues are conveyed through pulse rate. Combined opto-electrical stimulation paradigms implemented.

Results: Benchtop testing of the device was successful. The size and mechanical properties of the electrodes were at the upper acceptable limit. Sensitivity and dynamic range of the microphone were in the target range. Power consumption of the device, together with the available batteries, allows an 8-hour use of the device before recharging.

Conclusions: A prototype for the CI is on hand for performance testing in guinea pigs. The future test will use a full version of the implant to test in a preclinical trial in sheep. Focus will be on oeCI's performance, casing, immune response, and optrode placement. This study will assess the implant's biocompatibility, durability, and functions for future clinical implementations.

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T30. Cochlear Tropism of Murine Cytomegalovirus and Mumps Virus

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Background: Cytomegalovirus (CMV) is the most common cause of congenital sensorineural hearing loss (SNHL). CMV affects over 1 in 200 live births in developed countries, and over 10% of the cases develop unilateral or bilateral SNHL. The mumps virus (MuV) is a leading cause of acquired SNHL. Although vaccination has substantially reduced the incidence of MuV infection, it is estimated that 4-7% of sudden SNHL cases are caused by MuV. Despite the clinical significance of virus-induced SNHL, basic research in this area remains limited. As an initial step in our endeavor in this field, we aimed to make an atlas of viral infectivity in the cochlea and to gain a better understanding of its etiology.

Methods: Murine CMV (mCMV; Smith strain) and MuV (Odate strain) fused with GFP were propagated and titrated. For mCMV infection, Balb/c mice were used, whereas interferon-alpha/beta receptor (IFNAR) knockout mice were used for MuV infection. Whole cochlear explants were prepared at postnatal day (P2) and P21 to model congenital and acquired hearing damage caused by mCMV or MuV, respectively. The cochlear explants were infected with mCMV or MuV in culture to assess viral infectivity and cellular damage in various cell subtypes under conditions with minimized immune responses.

For in vivo experiments, infections with mCMV and MuV were performed at P2 and P21. mCMV was administered via the posterior semicircular canal (PSC) or intraperitoneally, while MuV was administered via the PSC. Viral tropism for cochlear cell subtypes was examined by immunohistochemistry, and auditory function following infection was assessed using auditory brainstem response (ABR).

Results: In vitro experiments demonstrated that mCMV infected spiral ganglion neurons but not the organ of Corti, consistent with previous reports. Histological analyses of in vivo-infected cochlear samples are currently ongoing and are being compared with the in vitro findings. Auditory brainstem response (ABR) performed at P32-40 after mCMV infection showed pronounced threshold shifts at low frequencies. In MuV-infected cochleae, preliminary observations indicated hair cell damage.

Conclusions: This study presents our initial steps to understand virus-induced hearing loss and cochlear pathology. We plan to expand the project to systematically examine viral tropism in individual cochlear cell subtypes, with particular focus on identifying the stages in the viral life cycle that critically control infection in specific cell types.

T31. Cochlear Neural Degeneration is Key to Word-Recognition Ability Among People With Similar Audiograms

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Background: Across a large audiological dataset (> 80,000 patients), those with Ménière's disease (MD) showed lower threshold-adjusted word-recognition scores (WRSs) relative to age-related hearing loss (ARHL) or other etiologies of sensorineural hearing loss (SNHL) [Grant et al., 2022, Sci. Reports 12(1):8929]. We hypothesized that cochlear neural degeneration is a key driver of these WRS differences among patients with similar audiograms. To test it, we quantified histopathology in human temporal bones with hearing loss from several common etiologies and assessed the contribution of each cell type to word scores in a multivariable linear regression.

Methods: We examined 136 human cases from the Mass Eye and Ear archive: those with MD (n=44), contralateral (unaffected) ears of those with MD (n=10), age-matched controls (n=44), cases with platinum ototoxicity (cisplatin/carboplatin; n=10), or aminoglycoside ototoxicity (n=13), and age-matched controls for this (younger) group of ototoxic cases (n=15). Mean ages were 81.36, 82.8, 75.5, 65.44, 69.31, and 64.47 years, respectively (80 males/56 females overall). All cases had recent audiograms; WRSs were available for 113/136 ears. We quantified fractional survival of inner (IHC) and outer (OHC) hair cells from H and E-stained slide sets that include every 10th section (20 µm). Auditory-nerve (AN) peripheral axons were counted in fluorescently labeled sections from each half-turn where the osseous spiral lamina is cut in cross-section. Strial area was measured at 14 cochlear locations, and spiral ganglion neurons (SGNs) were counted in all sections from MD cases their controls using machine learning.

Results: Of all groups, MD ears showed the lowest survival of IHCs (< 50%), OHCs (< 30%), AN peripheral axons (< 30%), and stria (< 60%). Correspondingly, the MD group had the worst mean thresholds (75.4 dB). Mean WRSs were also poorest in the MD group (36.68%), followed by platinum ototoxicity (62.75%), with best WRS in ototoxic controls (87.8%). Pairwise correlations showed the strongest WRS associations with IHC survival in the apical half ($R^2=0.404$) and with ANFs in both apical ($R^2=0.329$) and basal ($R^2=0.324$) halves. Despite the massive loss of AN peripheral axons, SGN loss re age-matched controls was minimal except in the apical 10%. In a multivariable regression including all histological variables and PTA (0.5–4 kHz), ANFs were the only significant histological predictor of WRS.

Conclusions: As predicted, MD cases exhibited the most widespread cochlear pathology and the poorest word scores among the cohorts studied. WRS deficits in MD were driven chiefly by neural loss once PTA had been accounted for; additional contributions from IHC, OHC or stria damage were not significant. The neural degeneration was seen as loss of peripheral axons from surviving SGNs, which explains why MD patients can perform well with cochlear implants.

T32. Impact of Reconstructed Wall Thickness on Postoperative Aural Fullness in Sigmoid Sinus Resurfacing: Clinical Value of Intraoperative Doppler Ultrasound

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Background: Venous pulsatile tinnitus (PT) often arises from sigmoid sinus wall anomalies and can be effectively treated with sigmoid sinus wall resurfacing surgery (SSWR). While SSWR provides favorable tinnitus outcomes, postoperative complications such as aural fullness remain a clinical challenge. In this clinical study, the optimal thickness of reconstructed sigmoid sinus wall and the role of intraoperative Doppler ultrasound (IDU) were investigated.

Methods: We retrospectively analyzed 35 patients who underwent SSWR between 2019 and 2023. Patients were divided into two groups according to IDU use (IDU group, n=6; non-IDU group, n=29). Postoperative reconstructed wall thickness was quantitatively measured on CT scans. Tinnitus severity was evaluated with the Tinnitus Handicap Inventory (THI) and Visual Analog Scale (VAS) at baseline, 3 months, and 6 months postoperatively. Logistic regression and ROC analyses were used to assess the impact of reconstructed wall thickness on postoperative aural fullness.

Results: Both groups demonstrated significant improvement in THI and VAS scores at 3 and 6 months, regardless of IDU use ($p < 0.001$). However, postoperative CT demonstrated that reconstructed walls were significantly thicker in the non-IDU group than in the IDU group (4.45 mm vs. 2.98 mm, $p < 0.05$). Persistent aural fullness occurred more frequently in patients with reconstructed wall thickness ≥ 5.0 mm. Logistic regression confirmed that increased wall thickness predicted higher risk of aural fullness (OR per 1 mm = 1.87, 95% CI 1.01–3.49, $p=0.047$). ROC analysis identified 5.0 mm as the optimal cutoff (AUC=0.72), with patients exceeding this threshold showing a markedly increased risk (OR=13.2, $p=0.006$).

Conclusions: SSWR is highly effective in alleviating tinnitus severity. However, excessive wall reconstruction (≥ 5.0 mm) significantly increases the risk of persistent postoperative aural fullness. IDU facilitates precise localization of sigmoid sinus anomalies, minimizes unnecessary cement application, and enhances postoperative comfort without compromising tinnitus relief. Incorporating IDU into SSWR therefore improve both clinical efficacy and postoperative aural fullness.

T33. Molecular Markers of Pediatric Cholesteatoma: A Bulk RNA Sequencing Comparison With Adult Tissue

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Background: This study aims to characterize the mechanisms responsible for the increased aggressiveness and higher rates of recurrence seen in pediatric cholesteatoma compared to adult cholesteatoma tissue samples using bulk RNA sequencing.

Methods: RNA sequencing was performed on 3 pediatric and 3 adult cholesteatoma tissue samples. Differentially expressed genes were identified using DESeq2, providing insight into the specific up- or downregulation of individual genes. Gene set enrichment analysis was performed using three genetic reference packages to identify gene sets that were up- or downregulated.

Results: Bulk RNA sequencing of 3 pediatric and 3 adult cholesteatoma tissue samples identified 20,298 genes. Differential gene expression analysis revealed 14 genes: 6 downregulated in pediatric compared to adult cholesteatoma and 8 upregulated in pediatric cholesteatoma compared to adult tissue. The gene set enrichment analysis revealed 3 gene sets upregulated in pediatric samples compared to adults relevant in cholesteatoma literature: Tumor Necrosis Factor (TNF- α), Transforming Growth Factor Beta (TGF- β), and the Epithelial-Mesenchymal Transition (EMT).

Conclusions: Gene expression differs between pediatric and adult cholesteatoma tissue samples, suggesting that the aggressiveness of pediatric cholesteatoma may be related to differential gene and pathway expression. This study highlights the complex inflammatory process seen in cholesteatoma and suggests that the molecular markers identified in this study could serve as potential drug targets in addition to surgical removal.

T34. Serum Biomarkers Affecting Hearing Loss in Neonates

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Background: Neonates admitted to the neonatal intensive care unit (NICU) have at a high risk of having or developing hearing loss. Risk factors for hearing loss are well documented in UK and US clinical practice guidelines. However, fewer studies report on potential serum biomarkers of hearing loss in neonates even though their serum biomarkers are regularly recorded. Hyperbilirubinaemia is a risk factor for hearing loss and identified in US guidelines. Serum bilirubin is a candidate serum biomarker for hearing loss as it can have neurotoxic effects on auditory brainstem nuclei. Damage to the auditory system occurs primarily within the brainstem and cranial nerve VIII. Concentrations of albumin are also of interest as neurotoxicity is caused by the fraction of unconjugated bilirubin not bound to albumin.

Aim: To identify modifiable biomarkers of hearing loss in the serum of neonates.

Methods: Chart reviews were conducted on the caseloads in NICUs of two UK hospitals and data for 142 neonates extracted. Cases were 71 neonates who were later identified as having hearing loss. Bilateral hearing loss (BHL) n=46 and 25 with unilateral hearing loss (UHL). BHL cases had a median gestational age of 32 weeks (range = 24-41 weeks), a median birthweight of 1370g (range = 521-3650g) and a median NICU stay of 44 days (range = 1-152 days). All hearing loss cases were individually matched with controls on gestational age, birth weight and sex. The median NICU stay of controls was 25 days (range = 1-131 days). Type and degree of hearing loss was determined from their most recent audiological tests performed by a paediatric audiologist.

First 5-day average, peak and trough serum concentrations of total serum bilirubin, conjugated bilirubin and albumin concentration were recorded during the NICU stay. Association between total serum bilirubin and serum albumin concentrations were assessed, and their association with gestational age and chronological age. Differences in serum biomarker levels between cases and controls were compared using Mann-Whitney U tests. Sensitivity and specificity were assessed using Receiver Operating Characteristic (ROC) curve analyses.

Results: Peak total serum bilirubin and trough albumin serum concentrations were statistically different between BHL cases and matched controls. Cases with BHL had higher levels of peak total serum bilirubin (240 $\mu\text{mol/L}$) than controls (166 $\mu\text{mol/L}$) (Mann-Whitney U = 475; $p=0.002$), and BHL cases had lower levels of minimum albumin (18 g/L) than controls (23 g/L; Mann-Whitney U = 338; $p=0.0001$). Following ROC analyses, area under the curve was 0.8 for albumin and 0.7 for bilirubin.

Conclusions: Peak total serum bilirubin and minimum albumin concentrations are candidate serum biomarkers for BHL in children admitted to NICU. The association between serum bilirubin and albumin concentration and treatment interactions are key in determining candidacy for hearing loss biomarkers.

T35. Cochlear Implantation in Children With Autism Spectrum Disorder Versus Neurotypical Peers

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Background: Cochlear implantation (CI) is a well-established intervention for children with severe-to-profound sensorineural hearing loss, leading to significant gains in speech perception, expression, and communicative development in neurotypical populations. Although additional disabilities were once considered a contraindication, research now demonstrates meaningful benefits across auditory, behavioral, and communicative domains. The outcomes in children with autism spectrum disorder (ASD) are more difficult to evaluate accurately, and their average gains tend to be smaller compared to neurotypical peers. While prior studies have compared CI outcomes between children with ASD and neurotypical controls, none have systematically evaluated the clinical, developmental, and rehabilitative factors underlying these differences in a matched cohort design.

Methods: Forty pediatric cochlear implant recipients (20 with ASD, 20 neurotypical) were retrospectively

identified and were included in this study. Groups were matched for sex, age at implantation, and

year of implantation to minimize confounding factors. Data was extracted through chart review, including age at implant, age of autism diagnosis, device use (datalogging, hours/day), speech therapy, ASD severity (classified per DSM-5 criteria), comorbidities, and follow up duration. Planned follow-up includes administration of a communication and behavior questionnaire to the patients in the cohort. Quality of life of both the children and parents will be assessed using validated instruments.

Results: Both groups improved post-implantation; however, neurotypical children had higher rates of

significant gains in speech expression (80% vs. 30%, $p=0.004$) and perception (85% vs. 50%, $p=0.047$). Among children with ASD, communication outcomes were significantly associated with ASD severity, comorbidities, and modifiable factors including device use, age at implant, and receipt of consistent speech therapy. Children with ASD were four times as likely to have average device use < 6 hours/day and twice as likely to lack consistent speech therapy. In fully adjusted models, ASD diagnosis remained significantly associated with poorer speech perception outcomes ($p = 0.011$) but not with speech expression ($p = 0.087$), suggesting that modifiable clinical and rehabilitative factors partially account for group differences in expressive outcomes but not in perceptive outcomes.

Conclusions: Children with ASD show reduced speech gains post-implantation compared to neurotypicals,

largely influenced by modifiable factors. These findings support the need for personalized rehabilitation to address those factors and ASD-specific quality of life assessment. This study emphasized the need for dedicated speech perception and expression scales and ASD-specific quality of life instruments to appropriately evaluate outcomes after cochlear implantation in this population.

T36. Co-MAP – The Cochlea Multi-Omic Atlas Project; an Integrative Multi-Omic and Spatial Atlas of the Developing Cochlea

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Background: Recent breakthroughs in single-cell and spatial omics technologies now allow unprecedented resolution of complex tissues. The cochlea, with its striking cellular diversity and base-to-apex gradients, is uniquely suited for such approaches. In a collaborative trans-NIDCD effort, we are generating a multi-omic and spatial atlas of mouse cochlear development.

Methods: We chose CBA/CaJ mice, a strain that retains stable hearing across the lifespan. Using the 10x Genomics technology, this atlas spans critical developmental milestones from E12 through aging (> 1 year) and integrates single-cell/single-nucleus RNA-seq to capture gene expression, single-nucleus ATAC-seq for chromatin accessibility, and Visium HD for spatial transcriptomics. Data analyses were performed in R using Seurat, Signac, ArchR and ProjectR.

Results: Co-MAP 1.0 (release date Feb 7, 2026) includes transcriptomic, epigenomic and spatial transcriptomic data from E16, P1 and P30 mouse cochleae. The data were analyzed to identify all major cochlear cell types, identify cell type-specific markers and define a diversity of gene sets. Co-MAP profiles were structured in gEAR to allow for users to interrogate this multi-modality atlas without having to download the data or have knowledge in programming. The integration of these data in the gEAR with ProjectR allows users to project clusters from their own data onto the atlas to identify cell types in user defined datasets. Finally, we present additional analyses linking transcription activity to open and closed chromatin state using Signac and ArchR.

Conclusions: Co-MAP is the first comprehensive molecular map of the developing cochlea, made openly available through the gEAR portal, at set data releases, well in advance of publication. By enabling the community to explore and interrogate these data, this atlas provides a transformative resource to accelerate discovery, hypothesis generation, data validation, and foster collaboration in hearing research.

T37. Early Inner Ear Organoids Generate Spontaneous Calcium Transients in Otic Progenitor Tissue

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Background: During the early phases of inner ear development, spontaneous Ca²⁺ transients are thought to play a crucial role in refining the structure and function of the peripheral sensory epithelia and their downstream neural circuitry. Different sensory and non-sensory tissues exhibit these transients during different phases of inner ear maturation. We asked whether inner ear organoids (IEOs; 3D stem-cell-derived models of the inner ear) recapitulate these calcium dynamics in vitro.

Methods: We generated early-stage IEOs from a specialized hiPS cell line expressing two fluorescent reporters: mScarlet expressed under the Prph (peripherin) promoter to label peripheral neural elements, and ubiquitously expressed GCaMP6f to report Ca²⁺ transients. Starting at day 21 of organoid culture (corresponding to late week 3-4 in vivo), we performed longitudinal Ca²⁺ imaging every 5-10 days using standard confocal laser-scanning microscopy. Spontaneous Ca²⁺ activity was recorded under baseline conditions, and we catalogued the patterns of Ca²⁺ transients as the organoids matured. We also performed SHIELD clearing-based immunohistochemistry to correlate the calcium recordings with tissue organization, such as neurite extension, contact, and synaptic refinement. In ongoing experiments, we are also testing pharmacological agents (e.g., purinergic receptor manipulation and neurotransmitter blockers) to probe the mechanisms underlying these signals.

Results: Our organoids exhibited robust spontaneous Ca²⁺ signaling reminiscent of that shown in developing mouse inner ear tissue. As early as day 21, we observed occasional spontaneous

Ca²⁺ transients in individual cells. As development progressed (through days 25-35+), the frequency and complexity of Ca²⁺ activity increased. Notably, organoids showed coordinated calcium waves propagating across clusters of cells, interspersed with discrete oscillatory Ca²⁺ spikes in single cells. These Ca²⁺ “waves” often originated in focal regions and spread through the prosensory area, suggestive of intercellular communication.

Conclusions: To our knowledge, this is the first demonstration of spontaneous Ca²⁺ signaling dynamics in inner ear organoids (IEOs). These findings indicate that organoids recapitulate essential aspects of in vivo inner ear development, including the emergence of spontaneous Ca²⁺ spikes and propagating waves, reflecting intrinsic developmental programs and cell–cell communication similar to those in the developing mouse inner ear. Establishing that IEOs generate native-like spontaneous Ca²⁺ activity provides a powerful platform to study auditory and vestibular development, dissect the contribution of pathways such as purinergic signaling, and explore pharmacological or regenerative strategies to modulate inner ear circuit maturation.

T38. Tafa3-Cre; Atoh1fx/fx: A New Mouse Model for Inner Hair Cell Specific Deletion of Atoh1 in the Developing Cochlea

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Background: The mammalian cochlea contains a highly ordered array of inner and outer sensory hair cells (IHCs and OHCs) surrounded by several types of non-sensory supporting cells. To achieve this cellular diversity, sensory progenitor cells undergo a series of cell fate decisions governed by transcription factors, notably including Atoh1. Atoh1 is necessary and sufficient for hair cell differentiation, and null mutations in this gene result in the complete loss of hair cells. In the mouse cochlea, cellular differentiation begins around embryonic day 13 (E13) with the onset of Atoh1 expression in IHCs near the base. Atoh1 expression then rapidly expands towards the apex over the next few days of development. As this occurs, Atoh1 expression also broadens across the medial-lateral axis, turning on in developing OHCs just after IHCs at equivalent apical-basal positions. It has been hypothesized that initial Atoh1 expression in IHCs is necessary for lateral expression of Atoh1 and therefore induction of OHCs, but this has not been tested directly.

Methods: Tafa3 (formerly Fam19a3) encodes a small secreted peptide and is specifically expressed in IHCs beginning at E14. The onset of Tafa3 expression makes it among the earlier markers of this cell population. We had previously generated mice null for Tafa3 and found that they were viable, fertile, and had normal hearing and inner ear anatomy, making Tafa3 a good candidate to drive IHC-specific Cre expression without confounding effects of Tafa3 loss-of-function. Using CRISPR, Cre was inserted into the first coding exon of the endogenous Tafa3 locus. We are using Tafa3-Cre and a conditional allele of Atoh1 (Atoh1-fx) to delete Atoh1 specifically in IHCs and will analyze the phenotype of this conditional loss of Atoh1 function.

Results: Using the R26-mTmG reporter line, we found that Tafa3-Cre faithfully recapitulates Tafa3 expression in the developing cochlea. By P1, nearly all IHCs were GFP+, indicating Tafa3-Cre activity, with only a few (< 10) OHCs labeled per cochlear duct. IHC-specific Cre

expression within the auditory system persisted into adulthood, and very little reporter expression was observed in other cell types within the inner ear, though Cre activity was evident in many other tissues, including the brain, spinal cord, kidney, and testis. Importantly, hearing function in Tafa3-Cre/Cre mice was comparable to littermates, as assessed by ABR threshold and DPOAE amplitude. We expect to report preliminary results of the IHC-specific loss of *Atoh1* in Tafa3-Cre, *Atoh1*-fx/fx cochleae.

Conclusions: We have generated a new mouse Cre line, Tafa3-Cre, which is specifically expressed within the cochlea in IHCs, beginning very early in HC differentiation. We have generated an IHC-specific deletion of *Atoh1*, which should help clarify whether *Atoh1*-mediated formation of IHCs is necessary for OHC development and subsequent formation of the cochlear sensory epithelium.

T39. Fgfr3-Etv Signaling Regulates Inner Pillar Cell Development in the Cochlea

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Background: The development of the organ of Corti requires precise specification of supporting cell subtypes. Among these, Inner pillar cells (IPCs) are critical for sensory epithelium architecture of the sensory epithelium and the formation of the tunnel of Corti. IPC development depends on Fibroblast growth factor receptor 3 (*Fgfr3*), activated by FGF8 from inner hair cells. Loss of *Fgfr3* abolishes IPCs, disrupting cochlear patterning and causing deafness. The ETS transcription factors *Etv1*, *Etv4*, and *Etv5* are recognized downstream mediators of FGF signaling in multiple organ systems. We recently found that *Etv1/4/5* triple knockout mice exhibit IPC defects, implicating these factors in cochlear development. To determine whether *Fgf8*-*Fgfr3* signaling similarly regulates *Etv* expression in the developing cochlea, we examined the expression of *Etv* genes in *Fgfr3* mutants. In addition, we used single-cell RNA sequencing of *Fgfr3* mutants to identify other IPC-specific genes regulated downstream of FGFR3.

Methods: We analyzed the expression of *Etv1*, *Etv4*, and *Etv5* using RNAscope on cochlear sections from *Fgfr3*^{-/-} and *Fgfr3*^{+/-} mice at embryonic days E14, E16, and E18. For transcriptomic profiling, cochleae from *Fgfr3*^{-/-} and *Fgfr3*^{+/+} littermates were dissociated into single cells and processed using the 10x Genomics Chromium Single Cell 3' platform according to the manufacturer's protocol. Data were analyzed using Seurat (v4.4.0). Differential gene expression between control and knockout samples was assessed using a pseudobulk approach, focusing specifically on cells within the prosensory domain.

Results: Cochlear expression patterns for *Etv1*, 4, and 5 were similar in *Fgfr3* mutants and controls at E14. However, all three *Etv* genes were significantly down-regulated in inner pillar cells (IPCs) of *Fgfr3*^{-/-} mutants at E16 and E18, particularly in the basal cochlear region. The normal expression pattern of *etv* expression at E14 suggests that *Fgfr3* is necessary for the maintenance, but not the initiation, of *Etv* expression in IPCs. Single-cell RNA sequencing (scRNAseq) at E16 revealed a marked reduction in the number of IPCs in *Fgfr3*^{-/-} cochleae, along with near-complete loss of expression of four IPC-specific *Etv* target genes. Broader changes in gene expression in other cochlear cell types were not observed indicating that *Fgfr3* signaling is restricted to IPCs. Pseudobulk analysis identified 13 significantly down-regulated

genes in *Fgfr3*^{-/-} cochleae, nine of which were IPC-specific. In situ hybridization confirmed loss of expression of IPC specific genes, including *Npnt* and *Lypd6*, in *Fgfr3*^{-/-} cochleae. Together, these results demonstrate that *Fgfr3* is critical for sustaining *Etv* signaling, IPC identity, and expression of novel downstream regulators of pillar cell development.

Conclusions: Our findings establish that maintenance of *Etv* signaling in IPCs by *Fgfr3* is required for IPC development. Moreover, the identification of novel IPC-specific downstream targets is the first step in revealing the previously unknown molecular network underlying pillar cell differentiation.

T40. Reduced or Absent Pou4f3 Expression Impairs Utricular Development via Direct Effects on Hair Cells and Non-Cell Autonomous Effects on Supporting Cells

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Background: Pou4f3 is a transcription factor that is first expressed in hair cells (HCs) during their early development. Because of its HC-specific expression pattern in the inner ear, Pou4f3 was chosen as the insertion site for the human diphtheria toxin receptor transgene, which yielded the Pou4f3DTR mouse line. Heterozygous Pou4f3DTR mice have been used extensively in the hearing field to selectively ablate HCs after administration of diphtheria toxin. The homozygous Pou4f3DTR/DTR mouse has also been used as a model of Pou4f3 deletion. Interestingly, recent work has shown that, unlike in the cochlea, some HCs persist in the vestibular epithelia of Pou4f3 mutant mice. In this study, we aimed to characterize utricles from Pou4f3DTR/DTR and Pou4f3^{-/-} using histological and transcriptomic methods.

Methods: Young adult mice (P28-P30) from two different Pou4f3 mouse lines were used in this study. The Pou4f3DTR mouse line was provided by Dr. Ed Rubel (University of Washington), while the Pou4f3^{-/-} mouse line was obtained from Dr. Tudor Badea (NEI). Utricles from homozygous mutants, heterozygous littermates, and wildtype littermates were collected and fixed for histology and in situ hybridization. Additionally, utricles were dissected, dissociated, and processed for single-cell RNA sequencing using the 10X Chromium v3 pipeline. Reads were normalized using SCTransform v2 and integrated using RPCA. Subsequent bioinformatics analyses were performed using Seurat.

Results: In both homozygous Pou4f3DTR/DTR and Pou4f3^{-/-} mice, utricles had smaller sensory epithelia and fewer HCs than controls. However, Pou4f3^{-/-} mutants had smaller utricles and fewer HCs than Pou4f3DTR/DTR mutants. HC nuclei in Pou4f3DTR/DTR utricles were immunoreactive for POU4F3, suggesting the presence of at least some portion of the POU4F3 protein. Single-cell sequencing indicated that Pou4f3DTR/DTR and Pou4f3^{-/-} utricles did not contain HCs with Type 1-like transcriptomes. Instead, all Pou4f3 mutant HCs clustered with Type 2 HCs from control animals, consistent with previously reported histological findings. Additionally, the Pou4f3 mutants did not appear to have striolar Type 2 HCs, and their supporting cells (SCs) exhibited altered gene expression relative to those of controls.

Conclusions: Pou4f3 mutants possessed smaller utricles with fewer HCs, suggesting that normal HC development of early-differentiating HCs is necessary for continued utricular growth. We found that the Pou4f3DTR/DTR mutant has a less severe phenotype than the Pou4f3^{-/-} mutant, likely due to its expression of at least part of the POU4F3 protein. Additionally, the SCs both Pou4f3 mutants exhibited altered transcriptomes, suggesting that there is a non-cell autonomous effect when Pou4f3 is disrupted. One possibility is that lateral inhibition, or some other intercellular signaling process, is affected when HCs fail to develop normally, resulting in a reduction in cellular proliferation and early arrest of postnatal growth in the utricle.

T41. Sensory Experience During the Critical Period Enhances Presynaptic Release at the Mouse Calyx Terminals

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Background: Activity-dependent synaptic plasticity shapes neural circuits in the developing brain. In the auditory system, sound deprivation or congenital deafness have been known to impair synaptic properties. However, the mechanisms by which enriched sound experience tunes synapse and circuit function during postnatal development remains poorly understood. We examined the mouse calyx of held–medial nucleus of the trapezoid body (MNTB) synapse, a model of fast, high-fidelity glutamatergic transmission, to define presynaptic mechanisms engaged by sound experience during early postnatal development.

Methods: We exposed mice to sound enrichment (3 hours per day, 80 dB broadband noise centered at 16 kHz) for 7 days from postnatal day (P)13 to P19. Using whole-cell patch-clamp recordings from the calyx-MNTB neuron synapses, we quantified short-term plasticity and presynaptic properties including paired-pulse ration (PPR), release probability (Pr), and the readily releasable pool (RRP).

Results: Sound enrichment (7days) resulted in a significant decrease in PPR and enhanced short-term depression, and an increase in presynaptic Pr, consistent with strengthened release at this synapse. To determine whether sound–evoked synaptic plasticity changes are associated with presynaptic excitability, we assessed presynaptic action potential waveforms at calyx terminals from control and sound enrichment group. There was no significant difference in amplitude, half-width, or threshold of action potentials, indicating presynaptic excitability is not a driver. Instead, presynaptic Ca²⁺ handling were affected: expression of calcium–binding protein, Calretinin, was significantly increased after enrichment, suggesting modified Ca²⁺ dynamics or buffering near release sites that could elevate Pr and reshape short-term plasticity.

Conclusions: Together, these findings identify a critical developmental period during which sound experience selectively enhances presynaptic release at the calyx of held. The data point to presynaptic Ca²⁺ regulation—not changes in spike waveform—as a key mechanism by which sensory experience refines auditory brainstem synapse and circuit function.

T42. Investigating the Role of Peripheral Tonotopy in Central Auditory Circuit Development: Insights From Bmp7-Induced Repatterning

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Background: Tonotopy, the spatial mapping of frequency processing in neural populations, is a key organizing feature of the auditory system. While tonotopy drives expression of anatomical and physiological specializations from the cochlea to central auditory structures, the role of peripheral tonotopic organization in development of central patterning remains poorly understood. In birds, a major functional role of the cochlear Nucleus Magnocellularis (NM) is to encode temporal information from acoustic input via phase-locked discharge output to the Nucleus Laminaris, the site of binaural coincidence detection. In order to preserve and improve phase-locking precision with respect to that observed in Auditory Nerve Fibers (ANF) across the tonotopy, ANF and NM neurons express physiological properties that are correspondingly tonotopically distributed. One prominent attribute is the distribution of synaptic terminal sizes of the ANFs onto NM somas. It has been shown that High Characteristic Frequency (HCF) NM neurons are innervated by one or two large 'end bulb' ANF terminals and Low Characteristic Frequency (LCF) NM neurons are innervated by multiple small synaptic boutons. Another tonotopic feature of NM neurons is the distribution of axon initial segment (AIS) lengths. LCF AISs are ~30% longer compared to HCF NM neurons (Kuba and Ohmori, 2009). In this study, we explored the effects of peripheral tonotopic repatterning on specialized features of NM circuitry, specifically focusing on how changes in peripheral tonotopy (BP) influences synaptic structure, size, and AIS length in the NM.

Methods: We manipulated tonotopic patterning of the inner ear by introducing the morphogen Bmp7 during the critical period for tonotopic differentiation in the embryonic chicken resulting in overrepresentation of LCF phenotypes across the tonotopic axis of the cochlea. To investigate the effect of peripheral tonotopic repatterning on ANF synaptic terminals, ANF fibers were orthogradely labeled with fluorescently tagged dextran injected into the ANF in vitro; AISs were immunolabeled with a marker for Ankyrin G. Synaptic NM terminal and AIS morphology was quantitatively analyzed across the tonotopic axis using syGlass software

Results: Preliminary evidence suggest that peripheral LCF overrepresentation paradoxically drives larger synaptic terminal development in HCF NM. However, we observed no modification of AIS length

Conclusions: Our data suggests that some features of central tonotopic specializations depend on patterned input from the inner ear

T43. Enhancer Activity Dynamics of Cochlear Hair Cell Maturation

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Background: Humans and other mammals do not regenerate hair cells after injury, leading to permanent hearing loss. The predominant strategy to induce mammalian hair cell regeneration is reactivating the developmental mechanisms that generate hair cells. Yet these approaches only regenerate immature, nonfunctional hair cells. These results suggest an incomplete understanding of the developmental mechanisms that produce mature, functional mammalian hair cells.

Elucidating mechanisms of hair cell maturation may improve regenerative therapies for hearing loss.

I aim to elucidate the role of enhancers in hair cell maturation. Murine cochlear hair cells mature over the first postnatal week; during this stage, the hair cells alter their gene expression profile and consequently gain mechanotransductive properties. Despite these changes, the enhancer activity dynamics of cochlear hair cell maturation are poorly understood. I hypothesize that immature, postnatal murine cochlear hair cells undergo changes in enhancer activity as they mature into functional sensory receptors. Broadly, I predict that dynamic enhancer activity modulates gene expression to promote cochlear hair cell maturation.

Methods: I analyzed publicly available 10x multiome datasets from murine P1 and P8 cochlear sensory epithelia (Iyer et al., Elife, 2022). Using Seurat and Signac packages, I clustered cells from both datasets. Next, I annotated P1 and P8 hair cell clusters by screening for known marker genes in the RNAseq gene expression data. I then used Signac to pseudobulk the ATACseq fragments and call chromatin accessibility peaks in P1 and P8 hair cell clusters. Next, I overlapped P1 and P8 hair cell peaks to reveal peaks that emerge and disappear during maturation.

Results: I assessed enhancer activity during maturation by comparing chromatin accessibility peaks between P1 and P8 hair cell clusters. I identified thousands of peaks with differential accessibility. 2234 peaks emerged from P1 to P8, and 8257 peaks disappeared from P1 to P8. Enhancer activity appears to be dynamic during cochlear hair cell maturation. These observations mark a step towards understanding how mature, functional cochlear hair cells form.

Conclusions: These preliminary results suggest that enhancer activity is dynamic during cochlear hair cell maturation. However, these data have caveats. The chromatin assay was hindered by low cell count, as hair cells cannot be easily obtained from P8 cochlea. To obtain larger hair cell counts for rigorous analysis, I will run 10x multiome on P0 and P5 cochlear sensory epithelia.

A basic understanding of enhancer activity dynamics in maturing hair cells will help decipher the gene regulatory mechanisms that drive hair cell maturation. Future directions include linking differentially accessible peaks to differentially expressed genes and running motif analysis on peaks. These results may reveal enhancers and their transcription factor binding partners that produce mature, functional hair cells. These factors could ultimately be used to enhance regenerative therapies for hearing loss.

T44. Single Cell Characterization of Utricular Supporting Cells

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Background: The mammalian inner ear consists of the cochlea, a spiral-shaped organ that confers our sense of hearing, and the vestibular system, which confers our sense of balance and acceleration. Our laboratory previously demonstrated that different subtypes of cochlear

supporting cells are transcriptomically distinct at the single-cell level, reflecting their different roles in trophic and structural support. By contrast, relatively little is known about supporting cell diversity in the vestibular epithelia. While vestibular organs have a simpler architecture by comparison with the cochlea, they contain several different types of hair cells, as well as different patterns of innervation between hair cells and vestibular ganglion neurons. This points to the possibility that the population of vestibular supporting cells may also include multiple unique subtypes. The aim of this project is the characterization of supporting cells in a vestibular organ, the utricle, over the course of postnatal development.

Methods: To generate single-cell transcriptional profiles of utricular support cells we used the 10X chromium platform to collect a total of 91,018 cells from mouse utricles dissected at 4 different timepoints; postnatal days 0-1, 5, 11-12 and 28-30. Transcriptional data was analyzed in R using Seurat v5. Quality control thresholds were set to remove low quality cells by filtering out contaminants, artifacts, and damaged cells. The data from each collection was normalized using SCTransform v2 for batch correction and variance stabilization. Datasets were integrated using layer-based, CCA integration.

To create a supporting cell dataset, hair cells, mesenchyme, neurons, glia and non-sensory epithelial cells were removed bioinformatically. The remaining supporting cells were then annotated and subset using canonical gene markers. Spatial localization of supporting cells in striolar and extrastriolar regions was biologically validated by RNA scope multiplex in situ hybridization.

Results: Single cell RNA-seq analysis of over 12,000 supporting cells identified 7 transcriptionally distinct clusters, each with a unique set of differentially expressed gene markers and enriched Gene Ontology (GO) terms. Four extrastriolar region cell subtypes, two striolar groups and one transitional epithelium cell type were characterized. We have biologically validated novel striolar (Cpa6-expressing) and extrastriolar (Scrg1-expressing) cell types using RNA Scope in situ hybridization. We are further validating our dataset by integrating with 10x Visium spatial transcriptomics data to produce a spatial map of supporting cell subtypes across the mouse utricle.

Conclusions: In this study we have profiled the molecular diversity of supporting cells in the mouse utricle across development, using high-throughput single cell RNA-seq and spatial transcriptomics. Our findings reveal broader transcriptional heterogeneity across supporting cell populations than was previously known, reflecting the multifunctional capacity of utricle supporting cells from phagocytosis to structural support to hair cell regeneration.

T45. Exploring Nrnx3 Postsynaptic Partners in Ribbon Synapse Formation

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Background: Ribbon synapses in hair cells (HCs) convert mechanical stimuli into electrical signals that are processed in the central nervous system. Previous studies have shown that the presynaptic cell adhesion molecules (CAMs) Nrnx3 plays a crucial role in the proper assembly of ribbon synapses in zebrafish and mice. However, the precise function of Nrnx3 in synapse assembly and the identity of its postsynaptic binding partners remain unclear.

Methods: We use the zebrafish lateral line to identify molecular components required for ribbon synapse formation. HCs in zebrafish share molecular similarities with those of the mammalian inner ear, making zebrafish a valuable model to identify novel molecules required for ribbon synapse formation. For our work we have used HCR RNA-FISH to determine the expression pattern of pre- and post-synaptic CAMs. We then apply these expression analyses and use CRISPR/Cas9 approaches to test whether CAMs contribute to the assembly of ribbon synapses.

Results: Using HCR RNA-FISH, we first evaluated splice isoforms of *nrnx3* mRNA in HCs. In particular, we assayed for the inclusion of the alternative splicing site 4 (SS4). The presence or absence of SS4 results in selective binding to distinct extracellular and postsynaptic CAMs. Our results show that for the two *nrnx3* paralogues in zebrafish (*nrnx3a* and *nrnx3b*), SS4 is absent in *nrnx3a* but present in *nrnx3b*. This indicates that each paralogue may have different binding partners. To decipher postsynaptic components, we assessed the expression of CAMs that are potential *Nrxn3* binding partners and found the presence of the orphan glutamate receptor D2 (*gluD2*) mRNA as well as neuroligin 3b (*nlgn3b*) mRNA in the soma of afferent neurons. We also observed high expression of the secreted molecules cerebellin 18 and 20 (*cbln18* and *cbln20*) mRNA in supporting cells. Previous work has shown that SS4 inclusion enhances affinity for *Cbln*, which in turn interacts with *GluD*, functioning as a modulator of excitatory synapses. Similarly, different *Nlgn*s exhibit variable affinities for multiple *Nrxn3* isoforms, both long and short, although in many systems these interactions remain unknown.

Conclusions: These preliminary results provide a starting point for deciphering the molecular mechanisms underlying synapse assembly during early development and may develop potential therapeutic strategies for synaptopathies associated with noise-induced and age-related hearing loss.

T46. Uncovering the Alternative Splicing Landscape of the Inner Ear During Development

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Background: Single-cell RNA sequencing (scRNA-seq) technology has demonstrated remarkable cellular heterogeneity and developmental dynamics in the mouse inner ear. However, transcriptional regulation is only one mechanism controlling gene expression. Alternative splicing is another key regulatory mechanism that increases the range of transcript diversity and proteins in a cell. Through alternative splicing, cells create structurally and functionally diverse proteins, modulate translational efficiency, control transcript localization, and produce non-coding RNAs. By understanding how mRNA isoforms are expressed during development, we can find more precise therapeutic targets and lower the risks of off-target effects. Previous studies investigating alternative splicing in scRNA-seq data were limited to pseudobulk approaches. We hypothesize that in addition to transcriptional heterogeneity, there is isoform level heterogeneity at the single cell level that governs diversity of cell types during

development. Here, we utilize the splicing Z score (SpliZ) to unveil the alternative splicing transcriptional landscape of the developing mouse inner ear at single cell resolution.

Methods: We utilized three publicly available scRNA-seq datasets (Kolla et al. 2020, Wilkerson et al. 2021, and Wang et al. 2024) focusing on the vestibular end organs (ampullae and utricle) and the cochlea. Specific timepoints and intervals investigated include: E14, E16, P1, P7 (cochlea), E14.5/E16.5, E18/P0, P5/P6, P12/P16 (utricle), and E16, E18, P3, P7 (ampullae). Quality control and cell type annotations were performed using known marker genes from the literature. We will apply the SpliZ algorithm, an annotation-free statistical method that quantifies alternative splicing events in droplet-based scRNA-seq data, to these datasets. Alternatively spliced genes specific to different cell groups within each organ and time point will be interrogated for statistical differences.

Results: For each of the three publicly available datasets, we were able to reproduce and annotate the broad cell clusters described. For the vestibular end organs, we focused our analysis on the epithelial and mesenchymal cell compartments. These include the hair cells, supporting cells, transitional epithelial cells, roof/dark cells, and the mesenchyme. For the cochlea, our annotation includes the greater epithelial ridge, lesser epithelial ridge, immature hair cells, inner and outer hair cells, Deiter cells, and pillar cells. We will report alternative splicing event differences among these groups of cells and expect to reveal both known and novel genes with alternatively spliced sites in the inner ear.

Conclusions: This study will uncover the regulated alternatively spliced single cell transcriptional landscape of the developing inner ear. Dissecting the complexity of mRNA alternative splicing in the inner ear may reveal new precise targets for gene therapies.

T47. Identification of Genes That Regulate Development and Maturation of Cochlear Supporting Cells

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Background: The sensory epithelium of the cochlea, the organ of Corti, is a highly derived and rigorously patterned structure that acts to convert auditory stimuli into electrical impulses. This structure consists of a highly diverse cellular mosaic that includes two different types of mechanosensory hair cells, and at least six different types of associated supporting cells. While there has been considerable research conducted on the development of hair cells, the maturation of supporting cells, which are equally necessary for normal hearing, has not been sufficiently examined. Therefore, this project aims to improve our understanding of supporting cell (SC) development by characterizing the maturation of pillar cells, Deiters' cells and Hensen's cells during the early post-natal period.

Methods: Cochlear duct cells were dissociated at postnatal day (P) 1, P3, P5, and P7 to obtain individual cells for analysis using single-cell RNA sequencing (scRNAseq) techniques. Bioinformatic approaches, including PySCENIC analysis, were employed to identify potential transcription factors that may modulate the development of different supporting cell types. To validate some of the cell-type-specific genes identified in the data set, we employed in situ

immunofluorescent labelling of mRNAs to visualize the localization of candidate transcripts within supporting cell types in cross-sections from postnatal cochlea.

Results: PySCENIC analysis of supporting cell scRNAseq data suggests that *Etv1* and *Etv4* regulons are highly active in inner pillar cells (IPCs) and outer pillar cells (OPCs) during the first postnatal week. This hypothesis is supported by localization of *Etv1/4/5* in inner and outer pillar cells at P1, P5 and P21. Furthermore, the PySCENIC analysis identified additional transcription factor candidates, such as *Gata2*, that may play crucial roles in SC development during the same time period.

Conclusions: This study presents a scRNAseq atlas for the development of supporting cells during early postnatal time points. The validation of these results indicates that these data will serve as a valuable resource for the examination of multiple developmental events that occur during the formation of the mammalian cochlea. As a first step in utilizing this resource, I plan to generate a conditional mouse line to disrupt the expression of *Etv* genes exclusively in postnatal SCs (*Sox2CreERT/+; Etv1fl/fl; Etv4-/-; Etv5fl/fl*). This approach was designed to ascertain the significance of these transcription factors in the postnatal survival and maintenance of IPC and OPCs. Similarly, a *Sox2CreERT/+; Gata2fl/fl* model will be established to examine the role of *Gata2* in the differentiation and maturation of lateral supporting cell subtypes.

T48. Gene Transfer of Transcription Factors Induces Hair Cell Regeneration in Mature Mammalian Cochleae

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Background: In ears with severe depletion of auditory hair cells (HCs), regeneration therapy may help restore some hearing, preserve neural substrates, and improve cochlear implant function. A combination of transcription factors (TFs) that play a role in hair cell (HC) development is also able to apply a HC phenotype on other types of cells in culture (Costa et al., 2015, Menendez et al., 2020). The aim of our work is to test outcomes of TF cocktails expression in supporting cells (SCs) in mature cochleae, with the goal of inducing their transdifferentiation into new HCs.

Methods: We tested a similar TF cocktail, *Gfi1*, *Atoh1*, *Pou4f3*, with or without *Six1* (GAP or GAPS) in mature animals in vivo, for its ability to induce HC regeneration. We used the *Pou4f3-DTR* mouse, where an injection of diphtheria toxin (DT) leads to degeneration of all cochlear HCs leaving behind SCs. DTR mice were deafened by injecting DT (25 ng/g). Adenovirus vectors containing gene inserts for GAP or GAPS were injected into the endolymph. The contralateral ear served as a control. One or two months later, cochlear whole-mounts were analyzed for presence of Myosin VIIa⁺ cells using epi-fluorescence or confocal microscopy.

Results: Both groups (GAPS and GAP) exhibited hair cell like-cells (HCLCs) in the experimental (left) ear. The GAPS group included 4 out of 10 animals with a large number of HCLCs. In the GAP group, 5 out of 10 animals had a large number of HCLCs. In both groups, HCLCs were located within the auditory epithelium and in ectopic locations.

Conclusions: GAP cocktail of TF transgenes appears to convert SCs to HCLCs in a similar way to the GAPS vector. The next important step is to transition the work to the AAV platform which is less toxic and more applicable for human clinical use.

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T49. Application of Inner Ear Gene Therapy to a Slc26a4 Mouse Model of Fluctuating Hearing Loss

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Background: Pendred Syndrome (PS) is one of the most common types of syndromic hearing loss, responsible for 5-10% of hereditary hearing loss cases. It is caused by mutations in SLC26A4, which encodes a transmembrane protein called pendrin. In addition to causing PS, variants in SLC26A4 have also been shown to cause enlarged vestibular aqueduct (EVA). Patients with EVA present with fluctuating and progressive hearing loss. Studies in the past have utilized knock-in and knockout Slc26a4 mouse models to further assess the role of SLC26A4 in hearing. However, these mutant mice have profound deafness instead of fluctuating hearing loss, which differs from the human phenotype. In this study, we investigate whether inner ear gene therapy can be used as a treatment for hearing loss in a doxycycline-inducible Slc26a4-insufficient mouse model with fluctuating hearing loss.

Methods: Adeno-associated virus serotype 8 (AAV8) packaged with Slc26a4 cDNA, a CMV promoter, and a GFP reporter was used to apply viral gene therapy into the inner ears of doxycycline-inducible Slc26a4 mutant mice. The expression of SLC26A4 in this mutant mouse model is controlled by doxycycline administration. It has been shown previously that if doxycycline is administered from conception and discontinued on embryonic day 17.5 (DE17.5), these mutant mice exhibit hearing instability. This phenotype is similar to patients with EVA. Inner ear gene delivery was performed between P0-P3 using a posterior semicircular canal approach. Auditory brainstem response (ABR) was recorded for four consecutive weeks, starting around P30, to assess the mice for hearing fluctuations. Endocochlear potential recordings were performed after P60. Inner ear tissues were then processed for immunochemistry and confocal microscopy imaging.

Results: The inner ears of DE17.5 mutant mice demonstrate minimal pendrin expression. After AAV8-CMV-Slc26a4-IRES-GFP treatment, pendrin expression was restored in the spiral

prominence, spindle cells of the stria vascularis, and endolymphatic sac. The treated DE17.5 mutant mice also showed a significant reduction in hearing fluctuation compared to non-treated DE17.5 mutant mice. Their EP also improved after AAV8-CMV-Slc26a4-IRES-GFP gene therapy treatment.

Conclusions: Our results suggest that AAV8-CMV-Slc26a4-IRES-GFP gene therapy restores pendrin expression in the inner ears of DE17.5 mutant mice. The gene therapy treatment also decreases hearing fluctuations and improves EP in the DE17.5 mutant mice. These results suggest that AAV8-CMV-Slc26a4-IRES-GFP gene therapy may be a viable treatment option for Slc26a4-related hearing instability.

T50. Transfection Efficiency of Vestibular Organs According to Various Aav Serotypes

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Background: Inner ear gene therapy, which involves the delivery of therapeutic DNA constructs to restore hearing and balance function, is an emerging field. Several synthetic adeno-associated virus (AAV) capsids have been developed and shown to provide higher hair cell transduction efficiency in the cochlea. While gene therapy for the cochlea has received significant attention, gene therapy for vestibular organs remains relatively underexplored. This study aims to provide a comparative analysis of different injection methods and AAV vector serotypes for gene delivery to the vestibular organs in both neonatal and adult mice.

Methods: First, we evaluated the efficiency of three distinct injection routes—posterior semicircular canal (PSCC), round window membrane (RWM), and scala media (SM)—in delivering AAV vectors to the utricle. CD1 wild-type neonatal mice (P0-P2) were injected with AAV9/PHP.B-CMV-eGFP. One week post-injection, the inner ears were harvested, and the percentage of transfected hair cells within the utricle was quantified using Cellpose 2.0 software. Next, we used the injection route with the highest transfection efficiency to test various AAV serotypes, including AAV9/PHP.B, AAV2.7m8, AAV2/Anc80L65, and AAV2/8, each carrying a CMV-eGFP reporter construct, in wild-type neonatal CD1 and C57Bl/6 mice.

Results: Our findings revealed that the PSCC route of injection yielded the most consistent delivery and highest transduction efficiency in the utricle, compared to the other injection methods. In subsequent experiments, PSCC injection route was used in neonatal and adult mice. When comparing various AAV serotypes delivered to neonatal mice, AAV9/PHP.B and AAV2/Anc80L65 demonstrated highest transfection efficiency in the hair cells of the utricle, saccule, and the ampullae of the semicircular canals.

Conclusions: AAV injection via the posterior semicircular canal appears to be the most efficient route for delivering gene therapy treatments to vestibular organs in mice. Both AAV9/PHP.B and AAV2/Anc80L65 were effective in transfecting hair cells within the vestibular organs. Further studies are necessary to optimize gene transfer strategies for adult mice. These results highlight the potential of PSCC delivery using AAV9/PHP.B and AAV2/Anc80L65 for future gene therapy applications targeting genetic vestibular disorders.

T51. Application of Inner Ear Gene Therapy to a Novel Myo7a Knockout Mice

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Background: Usher syndrome is a crippling disorder characterized by hearing loss, vision loss, and vestibular dysfunction. Worldwide, an estimated 4-17 per 100,000 people are affected by this disorder. Usher syndrome type 1b (USH1B) is the most common form of USH1, and it is caused by mutations in the unconventional motor protein MYO7A. In a previous study, we injected dual AAV8(Y733F) vectors carrying the two halves of the human MYO7A cDNA through the posterior semicircular canal (PSCC) approach in neonatal mice which led to improvements in vestibular function in the Shaker-1 (Myo7a^{4626SB/4626SB}) mice. When treatment was delivered in adult (P30) mice, we did not see any improvement in vestibular or auditory function. In this study, we explore the use of the dual AAV-PHP.B vectors to deliver the human MYO7A cDNA in neonatal and adult mice in a novel Myo7a knockout (Myo7a^{-/-}) mouse strain that exhibits haploinsufficiency similar to USH1B carriers.

Methods: Neonatal (P0-P3) and P30 Myo7a^{-/-} mice were used for this study. Dual AAV-PHP.B vectors were used to deliver the human MYO7A cDNA via the posterior semicircular canal into the inner ears of these animals. One month post injection, auditory brainstem responses (ABRs) were measured to assess auditory function, and vestibular evoked potentials (VsEPs) were recorded to evaluate vestibular function. Behavioral tests such as circling, rotarod, and swimming were also conducted to assess vestibular function. The inner ear tissues were collected and immunostained to evaluate viral transduction efficiency, sensory hair cell viability, and stereocilia morphology.

Results: Mutant Myo7a^{-/-} mice are profoundly deaf with vestibular dysfunction due to the absence of MYO7A expression. One month post dual-AAV-PHP.B-MYO7A injection, no recordable ABR responses were recorded. When vestibular function was assessed, P30 Myo7a^{-/-} mice showed improvement in VsEPs. Furthermore, behavioral testing in Myo7a^{-/-} mice showed significant improvement with the rotarod assessment after treatment when compared to untreated mutants. Immunohistochemistry showed MYO7A expression in both cochlear and vestibular hair cells of treated Myo7a^{-/-} mice.

Conclusions: The application of dual-AAV-PHP.B-MYO7A gene therapy restored MYO7A expression in both the cochlea and utricle and led to improvements in vestibular function in the Myo7a^{-/-} mice.

T52. Extending the Therapeutic Window: Ndp Overexpression Improves Hearing in an Adult Norrie Disease Mouse Model

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Background: Norrin, or Norrie disease protein (Ndp), is a secreted protein that functions as a Wnt pathway agonist, playing a critical role in cochlear vascularization, hair cell survival, and maintenance of the sensory epithelium. Mutations in Ndp cause Norrie disease, a rare, X-linked recessive genetic disorder characterized by congenital blindness, progressive hearing loss and cognitive decline. Our Ndp-KO mouse model recapitulates key features of human mutations, since it is not a complete null but reflects reported loss-of-function variants observed in patients. Previous studies have shown that Ndp overexpression can rescue hearing in neonatal Ndp knockout (Ndp-KO) mice, but whether this approach is effective beyond the early postnatal period remains unknown and is essential for defining the therapeutic window for intervention.

Methods: We used a conditional mouse model in which Sox2Cre drives tamoxifen-inducible Ndp overexpression (Ndp-OE) in an Ndp-KO background, enabling temporal control of gene activation in Sox2⁺ cochlear supporting cells. Sox2Cre; Ndp-KO; Ndp-OE mice were divided into treated (tamoxifen injection at postnatal day 30–35) and untreated groups; a control group consisted of Sox2Cre; Ndp-KO mice lacking the Ndp-OE construct. Cochlear function was assessed by auditory brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAE) performed 1 and 2 months of age to assess baseline hearing, progression, and post-treatment effects. Cell morphology, Ndp expression, hair cell integrity and vascularization were evaluated by immunohistochemistry on whole-mount cochleae and cochlear cryosections. Comparisons were made across wild-type mice, Ndp-KO mice, Sox2Cre; Ndp-KO mice and untreated and treated Sox2Cre; Ndp-KO; Ndp-OE mice.

Results: Through comparative review of published human NDP mutations, we determined that the Ndp-KO model reflects clinically reported loss-of-function variants rather than a complete null, establishing its relevance as a model for Norrie disease. ABR testing showed progressive threshold shifts across all frequencies in untreated controls between 1 and 2 months of age. In contrast, tamoxifen-induced Ndp overexpression significantly reduced threshold shifts at the basal-to-mid cochlear regions, where treated mice maintained stable or improved responses while untreated animals declined. Immunohistochemistry verified successful induction of Ndp in Sox2⁺ supporting cells and revealed improved cellular organization in the organ of Corti, including preserved hair cells. Vascular analyses compared differences between treated and untreated groups. Comparison with a viral-mediated Ndp delivery model demonstrated differences in expression patterns relative to the genetic overexpression approach.

Conclusions: These findings establish the Ndp-KO model as a clinically relevant representation of human NDP mutations and provide the first evidence that inducible Ndp overexpression at the young adult stage mitigates progressive threshold shifts. Together, these results support the feasibility of Ndp-targeted strategies for delaying or mitigating progressive hearing loss in Norrie disease.

T53. AAV-Mediated Exon Skipping Therapy for Usher Syndrome, Type 2A

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Background: Usher syndrome causes loss of vision, hearing and balance. There are four clinical subtypes, USH1 through 4, which are associated with mutations in genes important for structure, function and survival of photoreceptor cells in the retina and sensory hair cells in the inner ear. Genetic mutations in the USH2A gene, which encodes the protein, usherin, are the most common cause of Usher syndrome worldwide. The most frequent USH2A pathogenic variant is a single base pair deletion in exon 13, c.2299delG, that leads to a frame shift and expression of a truncated protein (p.Glu767Serfs*21). An investigational antisense oligonucleotide (ASO) for USH2A c.2299delG, QR-421a, designed to bypass the mutation, has already shown promise in Phase 1/2 clinical trials (Dulla et al., 2021). This ASO, designed to induce skipping of exon 13 to bypass the mutation, is suitable for patients who harbor at least one pathogenic mutation in that exon. While ASOs offer many advantages, they are sensitive to degradation and associated with a relatively short half-life. As such repeated injections may be required to maintain efficient therapeutic effect over long periods. To address this limitation, we created vectorized ASOs, taking advantage of uridine-rich 7 (U7) small nuclear RNA (snRNA).

Methods: We screened over 600 ASOs targeting exon 13 and its intron-exon boundaries to identify sequences that optimize exon 13 skipping. For this screen, we used a reporter construct designed such that a fluorescent tag would be only expressed upon restoration of the reading frame, i.e., upon exon 13 skipping. We selected the optimal ASOs, subcloned them into an U7snRNA plasmid and packaged them into AAV9-PHP.B or a AAV2-7m8 vectors. For validation, vectorized U7snRNA were tested in vitro on inner ear (IEOs) and retinal (ROs) organoids derived from a control human iPSCs line and USH2A c.2299delG KI line that we generated. RT-PCR was performed to validate exon 13 skipping.

Results: Several ASOs showed greater skipping efficacy than the QR-421a antisense sequence. We also noticed that combining two sequences in tandem led to better skipping efficiency. Using U7snRNA packaged into AAVs, we validated skipping efficiency with the novel antisense sequences in vitro in both IEOs and ROs.

Conclusions: Vectorized skippers offer a promising avenue for the treatment of USH2A with long term benefits that include a single local injection to prevent retinal degeneration and further progression of hearing loss in patients with deleterious mutations in exon 13.

T54. Local Administration of AAV to the Inner Ear via the Posterior Semicircular Canal Improved Sensorineural Hearing Loss in a Mouse Model of Norrie Disease

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Background: Norrie disease (ND) is an extremely rare X-linked recessive disease caused by pathogenic Norrie disease protein gene (NDP) mutations that result in defective norrin. ND patients typically present with congenital or childhood blindness, and although the majority pass newborn hearing screening, they develop progressive sensorineural hearing loss by their mid-20s (Sowden. 2020 Bryant. 2022). In this study, we attempted to treat NDP-KO mice by locally administering Adeno-Associated Virus Serotype1 (AAV1) containing normal NDP, or CHIR, an activator of the Wnt/ β -catenin signaling pathway.

Methods: Ndp-KO mice and CBA/CaJ mice were purchased from Jackson Laboratory. In the first set of experiments, Ndp-KO (y/-) male or Ndp-KO (-/-) female mice were randomly assigned to one of the 4 groups: untreated group, CHIR (40 mM) treated group, AAV1 (mouse NDP) treated group, and AAV1 (human NDP) treated group. In the second set of experiments, we crossed Ndp-KO mice of the C57BL/6 background with CBA/CaJ wild-type mice to create mice with restored cadherin 23 ahl alleles, which cause early-onset age-related hearing loss in the C57BL/6 strain, to confirm the long-term efficacy of AAV1 therapy. Male Ndp-KO (y/-) mice were randomly assigned to 2 groups: Untreated group and AAV1 (mouse NDP) treated group. CHIR or AAV1 was administered by local injection into the inner ear via PSSC at P2–6. At 1 and 2 months of age (and additionally at 4 and 6 M in mice with a half CBA/CaJ background), inner ear function was evaluated by ABR and DPOAE.

Results: In the analysis of inner ear function, particularly ABR thresholds, Ndp-KO mice showed progressive hearing loss at P30 and 60, as reported previously (Hayashi. 2021). The CHIR-treated group showed significant improvement at 5.66, 8.00, 11.32, 16.00, and 22.64 kHz At P30. The threshold increased slightly at P60, but significantly better hearing was still maintained. In contrast to the CHIR treatment group, no obvious increase in threshold was observed in the AAV1 (mouse NDP) or AAV1 (human NDP) treated group, and significantly better hearing was maintained at 5.66, 8.00, 11.32, 32.00, and 45.24 kHz at P30 and P60. 4 months had passed since the C57BL/6-CBA/CaJ background Ndp-KO mice were treated with AAV1 when this abstract was submitted, and significantly better hearing is maintained.

Conclusions: Recently, OTOF gene therapy has been clinically used in the field of otolaryngology and has shown good results (Wang. 2024). In our study, we achieved long-term favorable hearing outcomes by local therapy for Norrie disease. Our AAV1-based local gene therapy can be performed similarly to the OTOF gene therapy already being used clinically. Treatment with CHIR also showed temporary improvement in hearing loss. This result suggests that continuous administration of CHIR at regular intervals may prevent hearing loss caused by ND.

T55. Gene Therapy for DFNB6 Recovers Auditory and Vestibular Thresholds in a Tmiesr/sr Mouse Model

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Background: Transmembrane inner ear (TMIE) is an essential component of the mechanosensory transduction complex, which is responsible for sound detection. It is proposed that TMIE interacts closely with Transmembrane Channel 1 (TMC1), a mechanically gated ion

channel. Deficiencies in TMIE are associated with autosomal recessive deafness 6 (DFNB6), characterized by severe-to-profound non-syndromic sensorineural hearing loss. A therapeutic strategy for humans with mutations in the TMIE gene has not been developed. Fortunately, a mouse model of DFNB6 is available, spinner mice, which express a truncated version of TMIE that causes deafness, as well as spinning and hyperactive behavior.

Methods: P1-2 spinner mice (Tmiesr/sr) were injected with 1.1 µl of AAV-hTmie-GFP in the left utricles. Mice were tested for auditory brainstem responses (ABRs) and otoacoustic distortion products (DPOAEs) at 4, 8 and 12 weeks of age. 2 weeks later, mice were tested for vestibular evoked potentials (VsEPs). Lastly, we performed behavioral tests to compare their performance in an open field arena, RotaRod and PalmReader. Once all testing was concluded, cochleas were harvested and processed for immunohistochemistry. Some mice were not euthanized and instead were tested for ABRs and DPOAEs at 1 year of age.

Results: After viral injection, 4-week-old Tmiesr/sr mice presented an improvement of ~25 dB in their hearing thresholds across all frequencies, when compared to Tmiesr/sr uninjected mice. Furthermore, 1 year old Tmiesr/sr injected mice still maintained this improvement in their hearing thresholds. Regarding their vestibular function, VsEPs results were variable: 2 out of 5 injected spinner mice had VsEP thresholds similar to Tmie+/+ mice while 3 out of 5 exhibited no detectable thresholds (Tmie+/+ = -14.1 ± 0.8 g/ms, n = 15; Tmiesr/sr injected = -5.7 ± 3.9 g/ms, n = 5; p = 0.0019).

Additionally, RotaRod results showed a trend with Tmiesr/sr injected mice recovering balance (Tmie+/+ = 86.85 ± 14.5 sec, n = 8; Tmiesr/sr uninjected = 61.2 ± 23.5 sec, n = 4; Tmiesr/sr injected = 105.5 ± 12.7 sec, n = 3). Moreover, open field results showed that Tmiesr/sr injected mice exhibited a significant decrease in hyperactive movements when compared to Tmiesr/sr uninjected mice (Tmiesr/sr uninjected = 1809.4 ± 197.5 sec, n = 4; Tmiesr/sr injected = 752.5 ± 32.8 sec, n = 2; p = 0.0005). Results are expressed as mean values $\pm$ SEM. Statistical analysis was performed with Two-way ANOVA, followed by Tukey's multiple comparisons test.

Conclusions: AAV-hTmie-GFP mediated gene therapy in Tmiesr/sr mice can restore hearing and vestibular function. Although variable, some of the injected mice exhibited ABR, DPOAE and VsEP thresholds similar those of Tmie+/+ mice. Preliminary behavioral data suggests that viral injections can restore balance and reduce hyperactivity of spinner mice.

T56. Novel AAV-Capsids for Targeting the Auditory Nerve in Chronic Sensorineural Hearing Loss

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Background: Despite its success, users of electrical cochlear implants (eCI) report limitations in speech perception in daily situations with competing sounds. This is largely attributed to spread of electrical current in the cochlea, where each electrode activates a broad population of spiral ganglion neurons (SGNs) across a substantial frequency range. One solution to overcome this

issue is to stimulate the SGNs with light instead of electricity, as light can be better confined in space, promising better frequency selectivity. Recombinant Adeno-associated viral vectors (rAAVs) have been used to render SGNs light-sensitive by transgenic expression of light-activated ion channels known as Channelrhodopsins (ChR). Our group has provided in vivo evidence to support the clinical potential of the optical CI, but for clinical translation, it is critical to optimize the individual components of the gene therapy. Thus far, AAV variants tested in the inner ear derive from AAV library screens on other tissues or in silico design. Hypothesizing that high throughput in vivo selection screens of AAV libraries for variants with tropism for SGNs yields in better suited variants, we performed a multiplexed directed evolution approach.

Methods: Mongolian gerbils were deafened with kanamycin, and 4 weeks later a mix of AAV1, 2 and 6 libraries presenting a random unique 7-mer peptide inserts at variable region VIII of the capsid protein, were administered to the scala tympani via microcatheter inserted through the round window. After 24 hours post-administration, SGNs were harvested from cochleae (n = 10 animals per round). Viral genomes recovered from SGNs were PCR-amplified and capsid peptide sequences identified by Next Generation Sequencing. Variants enriched in SGNs were vectorized to be used as a refined expression library for subsequent rounds of screening.

Results: After two selection rounds, we found distinct variants that accumulated in SGNs alone, where up to 9% were AAV2-based. Eleven promising candidates were produced as vectors packaging eGFP that were equipped with a unique barcode and were pooled for a third screening round for highest expression in SGNs. While the analysis is currently underway, we expect to uncover variants with superior transduction efficiency compared to state-of-the-art AAV capsid. To demonstrate functional evidence for their superior efficiency, the rAAVs will finally be packaged with a ChR and delivered to the cochleae of chronically deafened gerbils, upon which optically induced auditory brainstem recordings will be collected.

Conclusions: This study provides support for the efficacy of SGN-targeted gene therapy and optogenetic hearing restoration in a rodent model of chronic, aminoglycoside-induced sensorineural hearing loss. We have identified rAAVs capable of overcoming barriers associated with a chronically deafened adult gerbil cochlea, allowing efficient transduction SGNs. Further work is needed to optimize all individual components of the optogenetic gene therapy with these rAAVs.

T57. Cortical Development After Bilateral Versus Unilateral Gene Therapy in DFNB9: An fNIRS Study of Neuroplasticity

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Background: Autosomal recessive deafness 9 (DFNB9) is a common genetic cause of congenital, severe-to-profound hearing loss in children, and has recently been restored in first-in-human gene therapy (GT) trials. It's unclear that how the laterality of auditory GT — unilateral

(left or right) versus bilateral — shapes early cortical development and functional network organization. We used functional near-infrared spectroscopy (fNIRS) to characterize the patterns of cortical activation and functional connectivity during speech and music processing after treatment.

Methods: In this prospective observational study, children with DFNB9 who received left-sided, right-sided, or bilateral GT were assessed at baseline and 4–6 weeks (T1), 13 weeks (T2), and 26 weeks (T3) after treatment. A 42-channel fNIRS system covering temporal, parietal, and frontal regions was used to measure event-related cortical activity by recording changes in oxygenated and deoxygenated hemoglobin (HbO/HbR). Auditory stimuli were presented in a block-design paradigm comprising two conditions: music and speech. Data preprocessing included optical-density conversion, motion-artifact correction, and band filtering. For each task block, activation level was quantified as mean HbO in a 5–10 seconds post-stimulus window. Functional connectivity was assessed using the multivariate Granger causality analyses to calculate correlation coefficients between HbO signals across all channels, quantifying inter-regional hemodynamic synchrony. Between-group comparisons used nonparametric tests.

Results: A total of 11 children with DFNB9 who received unilateral (left or right) or bilateral GT were enrolled. In both music and speech tasks and all time points, bilateral GT consistently elicited the largest cortical responses and the strongest interregional connectivity in the prefrontal cortex (PC), left/right hemisphere (LH/RH), superior temporal gyrus (STG), middle temporal gyrus (MTG), and Broca's area (All $p < 0.05$), exhibiting its broad advantage in network integration. For music, bilateral GT exceeded right-GT in 4 channels at T1 and T3 and exceeded left-GT in 5 channels at T1, with effects concentrated in RH, STG, MTG, and PC ($p < 0.05$). For speech, bilateral and right-sided GT consistently outperformed left-sided GT in STG, MTG and PC, with a developmental shift from early RH engagement to stronger LH (LH STG and Broca) specialization by T3; mean PC connectivity at T3 was significantly higher in the bilateral group than in both unilateral groups ($p < 0.05$). At T1, right-GT showed stronger interhemispheric (LH↔RH) coupling and enhanced RH→PC influences, whereas left-GT demonstrated relatively greater activation and connectivity in music-related regions ($p < 0.05$).

Conclusions: These preliminary longitudinal fNIRS data indicate distinct, laterality-dependent neuroplastic trajectories following GT in DFNB9. When feasible, bilateral intervention confers broader activation and network integration benefits; right-sided GT preferentially enhanced activation and interregional connectivity within speech-related networks, whereas left-sided GT music processing.

T58. Studying the Shared Genetic Association of TUBA1c to Sudden Deafness and Vestibular Neuritis

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Background: Sudden sensorineural hearing loss (SSNHL) is an otologic emergency, characterized by acute hearing loss of 30 dB in three contiguous frequencies. Potential causes include viral infections, cochlear artery occlusion, and biochemical changes. Histopathology often shows atrophy of the organ of Corti and striae vascularis. Treatment includes observation, steroid therapy, and various drug combinations. The degree of hearing loss varies among cases. Prognosis is influenced by the audiometric pattern and symptoms, with better recovery linked to the absence of vertigo and low tone hearing loss. Vestibular neuritis (VN), a common cause of peripheral vestibular vertigo, involves idiopathic inflammation of the vestibular nerve, causing vertigo, nausea, and imbalance. Symptoms typically resolve within weeks. In case that symptoms of SSNHL and VN persist over 3 months, an acoustic canal magnetic resonance (MRI) to explore possible etiology from central nervous system as acoustic neuroma (a benign brain tumor, known as vestibular schwannoma,) A missense variant close to TUBA1C has been linked to SSNHL and VN in Finland. However, the heritability of those conditions remains unclear. Evidence is very low, and linkage studies have not been published yet. Aims: The proposed project aims to study further the genetics and molecular mechanisms associated with SSNHL and VN.

Methods: Genome Wide Association Study (GWAS) for 3342 cases with vestibular neuritis and 3861 cases with sudden deafness. Expression of TUBA1c in the inner ear was studied in murine tissue and in 2D in vivo culture of human spiral ganglions using immunofluorescence (IF). The CodeWas data were analyzed to show which other events, medications, or diagnoses are common among cases of SSNHL and VN. LabWas data were analyzed to determine potential disease-associated laboratory tests.

Results: GWAS study in Finnish population for 3342 cases with vestibular neuritis and 3861 cases with sudden deafness and revealed shared loci in chromosome 12. Those loci included the recently reported missense variant rs200317762 close to TUBA1c. The IF showed the expression of TUBA1c in spiral ganglion neurons. Usage of betahistine and prednisolone were common among SSNHL and VN cases. A positive *Borrelia burgdorferi* IgM Ab [Units/volume] in Serum and high levels of N-Terminal Pro-B-Type Natriuretic Peptide (mean measured value was 1753.95 ng/l) were associated with SSNHL and VN.

Conclusions: Our results indicate that SSNHL and VN are genetically associated at least in some cases. Further studies are needed to explore the possible explanatory mechanisms.

T59. Discovering Age- And Sex-Specific Genetic Risk Factors in Sensorineural Hearing Loss: Genome-Wide Evidence From Large-Scale Biobank Studies

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Background: Investigate the genetic components of sensorineural hearing loss (SNHL) by performing genome-wide meta-analyses using the data from FinnGen and Estonian Biobank.

Methods: Genome-wide association study (GWAS) of SNHL cases in the general population and in sex- and age-of-onset stratified subgroups. The study-specific GWASs were combined through inverse variance-weighted genome-wide meta-analyses, encompassing a total of 531,059 individuals (Ncases=35,960). Age-stratified meta-analyses included 28,198 individuals diagnosed at the age of 55 years or after and 7,762 individuals diagnosed before the age of 55 years, with 495,099 controls. Sex-stratified meta-analyses included 313,501 females (Ncases=17,761) and 217,558 males (Ncases=18,199)

Results: In the meta-analysis focusing on the general population, 22 loci were observed, seven of which were previously unreported. In the sex-stratified analysis, two previously unreported loci were observed in the female subgroup and one locus in the male subgroup. In the age-stratified analysis, three previously unreported loci were observed in the subgroup of those that were diagnosed at the age of 55 years or after. In those with an earlier age-of-onset, one previously unreported locus was observed. Thus, 14 out of 32 reported loci have not been previously linked to SNHL. Differences in the effect sizes of the lead variants at each locus between the analytical subgroups were observed for 14 variants

Conclusions: Our study provide additional insight into the genetic underpinnings of SNHL. Our results validate the role of mechano-transduction and genetic components affecting the structure of the inner ear in the background of SNHL.

T60. Genetics of Prelingual Isolated Deafness and Usher Syndrome in the Maghreb and Jordan: Harnessing the Potential of Homozygosity

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Background: The molecular genetic diagnosis of prelingual sensorineural hearing impairment (HI) is essential for genetic counseling and patient management. Effective diagnosis requires a knowledge of the genetic architecture of HI, which is often lacking, especially in North Africa, with its consanguineous populations.

Biallelic missense mutations of MYO7A, CDH23, PCDH15, and USH1C cause either autosomal recessive isolated deafness (DFNB forms) or Usher syndrome type 1 (USH1) (USH1/DFNB genes). These defects are overrepresented in consanguineous populations, highlighting the need to be able to distinguish between the mutations causing these two types of conditions.

Biochemical studies have been unable to establish clear correlations between the changes in protein 3D structures caused by these USH1/DFNB gene mutations and the resulting phenotype: USH1 or DFNB. We hypothesized that these difficulties might stem from an incorrect or incomplete classification of certain mutations.

Methods: We established a cohort of 450 unrelated patients with familial (at least two affected relatives) severe-to-profound bilateral prelingual HI from five countries with high rates of consanguinity: Tunisia, Jordan, Algeria, Morocco and Mauritania (the TJAMM cohort). Genomic DNA from the patients was analyzed by a targeted gene panel sequencing strategy using HearPanel-IdA-1.

Results: Inheritance was recessive in 92% and dominant in 8% of cases and 14% of patients had syndromic deafness. Genome analysis detected 211 different mutations (36% of which were previously unknown) in 49 deafness genes, and fully resolved 90% of DFNB cases, in which 89% of the mutations were present in the homozygous state. The deafness genes involved were similar between countries but, with the exception of a few GJB2 and LRTOMT mutations, the mutations implicated differed considerably.

By studying patients homozygous for USH1/DFNB mutations, first in the TJAMM cohort and then for all 304 mutations reported in the homozygous state, we identified and characterized, among those previously characterized as causing DFNB, some mutations that underlie a gene dosage-sensitive USH1 phenotype, a pattern reported for recessive hypomorphic mutations. These mutations, unlike true DFNB mutations, were predicted to affect protein 3D-structure.

Conclusions: The genetic architecture of the TJAMM cohort suggests an overrepresentation of private mutations. The finding of USH1-hypomorphic like mutations extends the spectrum of conditions associated with defects of USH1/DFNB genes. It also paves the way for determining the type of mutations involved in patients compound heterozygous for two missense mutations, a common situation. This revised classification of USH1/DFNB missense mutations should provide a decisive contribution to efforts to establish the molecular criteria for predicting the presence/absence of retinal and vestibular abnormalities in patients. The detection of USH1-

hypomorphic-like mutations in patients should lead to thorough ophthalmological examinations throughout their lives. Altogether, this new classification has the potential to improve both the molecular diagnosis of DFNB and USH1 and patient management significantly.

T61. Nine Hundred and Counting; Hereditary Hearing Impairment Genes in Humans and Mice

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Background: Hearing impairment is a common, complex disease in humans with a high genetic contribution. It has been estimated that at least 1000 genes may underlie hearing impairment. Many have been reported in the literature and are listed on the Hereditary Hearing Loss Homepage, and many more candidates have been reported to affect hearing in mice, including genes from large-scale screens. Previous efforts to compile lists of genes underlying hearing have been extremely useful in research; here we present our compilation of hereditary hearing impairment genes in humans and in mice.

Methods: The list was based on published papers, published conference abstracts, and mouse phenotype data made available through the International Mouse Phenotyping Consortium (IMPC; <https://www.mousephenotype.org>). Each gene report was curated before inclusion. Associations were not included, and neither were genes which had only been investigated in combination with other mutations, such that it was unclear which gene was responsible for the auditory phenotype. Pathway analyses were carried out using Reactome. Expression analysis was carried out using data made available through the gEAR. The Hereditary Hearing Loss Homepage was used to identify loci for which no causative gene is yet known.

Results: The hereditary hearing impairment gene list consists of over 900 genes where there is good evidence that mutations result in affected auditory thresholds in humans, mice, or both. Reactome pathway analysis showed that genes from the list are found in a wide range of pathways, including Signal Transduction, Sensory Perception, and Development. Expression analysis showed expression in many different cell types of the inner ear. We analysed the positional data from human deafness loci for which no causative gene has been identified, and found several candidates from the list which merit follow-up, such as CASP3 and DCTD for DFNA24.

Conclusions: The complex array of different cell types which make up the ear, and the complexity of the molecular pathways required for its development, maintenance and function, mean that hearing impairment is a highly heterogeneous condition. Our list is useful for suggesting candidate genes for GWAS, sequencing, and family studies, and carrying out enrichment analyses on candidate gene lists. The list is available on Zenodo (<https://doi.org/10.5281/zenodo.15705587>), and will be updated periodically. Nominations and suggestions for genes to add are welcome.

T62. A Tale of Two Mice: The Role of Chd7 Dosage in Inner Ear Development and Function

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Background: Precise temporal and spatial gene expression is essential for proper development of the mammalian inner ear. Chd7 is a key regulatory component of the pathways that govern vestibular and auditory structures. Prior studies have shown that Chd7-haploinsufficiency and Chd7 deletion using tissue specific Cres (Pax2 or FoxG1) result in impaired inner ear development. Little is known about the effects of increased Chd7 dosage and whether it may rescue inner ear defects. Here we describe the generation and characterization of two novel mouse models; Chd7+/FAST to examine Chd7 dosage and rescue and Chd7+/CreERT2 to examine Chd7 lineage cells. Coordinated use of these alleles allows for rigorous studies exploring the timing and specificity of Chd7 functions in the ear.

Methods: Chd7+/FAST mice were engineered in collaboration with Ingenious targeting laboratories. The Flexible Accelerated STOP Tetracycline Operator-Knockin (FAST) cassette was inserted upstream of the ATG site in Chd7 exon 2. Chd7+/CreERT2 mice were developed in collaboration with the University of Michigan Transgenic Core using CRISPR/Cas9 technology to insert a CreERT2 recombinase cassette at the ATG site in Chd7 exon 2. Allele functionality was verified by crossing Chd7+/Gt and Chd7+/FAST or Chd7+/CreERT2 mice. Litters were collected at embryonic day 10.5 (e10.5) and stained with anti-CHD7 antibody. Crosses were established between Pax2cre and Chd7+/FAST mice to analyze e14.5 embryos by paint-fill. To assay Cre activity, Chd7+/CreERT2 mice were crossed with Sun1 reporter mice and pregnant dams injected with tamoxifen at various timepoints, followed by embryo harvesting at e10.5 or e18.5 and staining with anti-GFP. Inner ears were examined by crossing Chd7+/CreERT2 and Chd7Flox/Flox mice, injecting tamoxifen at various timepoints and analyzing embryos by paint-fill at e14.5.

Results: CHD7 antibody was absent in e10.5 Chd7 FAST/Gt or Chd7 CreERT2/Gt embryos, validating that Chd7 FAST and Chd7CreERT2 are null alleles. The inner ear was properly formed in paint-filled Pax2Cre;Chd7+/FAST embryos, confirming that restoration of Chd7 dosage in early development is sufficient to prevent inner ear malformations. Cre reporter (GFP) expression was present in the e10.5 Chd7+/CreERT2 otocyst after tamoxifen delivery at e8.5 or e9.5, signifying Cre activity in Chd7 lineage cells. GFP was not detected in the absence of tamoxifen, excluding spurious Cre activity. The e8.5 timepoint was identified as the most sensitive to reduced Chd7 dosage.

Conclusions: Chd7+/FAST mice exhibit heterozygous loss of Chd7 function and can be used for conditional restoration of Chd7. Chd7+/CreERT2 mice express Cre from the Chd7 locus, exhibit heterozygous loss of function, and can be used to trace Chd7 lineage cells. These novel mouse strains will provide important information about Chd7 dosage in inner ear formation and promote the design of therapies for hearing and balance disorders.

T63. IKZF2 is Necessary for OHC Maintenance and Regulates Pathways Required for Cell Membrane Asymmetry and Stereocilia

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Background: Transcription factors control expression of genetic networks that describe important checkpoints of cochlear development. In 2018, our laboratories identified the zinc-finger protein, Helios, as a necessary transcription factor for correct outer hair cell (OHC) maturation. Encoded by the *Ikzf2* gene, Helios transcripts were enriched in OHCs of mice from P4 to adulthood. Our mouse model, harboring a point mutation in *Ikzf2*, exhibited early-onset hearing loss and decreased OHC electromotility. Ectopic expression of Helios in inner hair cells (IHCs) revealed a transcriptional shift towards an OHC-like state. However, the gene regulatory network regulated by *Ikzf2* in OHC remained largely unknown. Here, we first determine if Helios also plays a role in maintaining OHC function after maturation using *Ikzf2* conditional knockout (cKO) mice. Next, a multi-omic and spatial transcriptomic approach was used to systematically delineate and validate the disrupted regulatory networks following *Ikzf2* deletion.

Methods: The dimerization domain of *Ikzf2* (Exon 8) was conditionally deleted by crossing *Ikzf2* floxed mice with *Gfi1*-Cre (deletion at ~E16.5) or *prestin*-CreERT2 (tamoxifen-induced at P12 or 6 weeks) mice. Auditory function of these *Ikzf2* cKO mice was evaluated at 6-weeks and 3-months of age by auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE) measurements. To quantify OHC loss, cytochrome c histochemistry was performed on cochlear preparations from 6-week-old mice, stained with phalloidin, DAPI and anti-prestin antibody. Annexin V staining was applied to harvested *Ikzf2*^{fl/fl};*Gfi1*-Cre cochleae to identify changes in cell membrane asymmetry. Stereocilia were evaluated using scanning electron microscopy (SEM). OHCs were isolated from P8 *Ikzf2*^{fl/fl};*Gfi1*-Cre cochleae using flow cytometry and analyzed through bulk and single cell RNA-seq. Transcriptomic results were validated using spatial transcriptomics and RNAScope. Helios-deficient OHCs were investigated for epigenetic changes using single nucleus ATAC-seq on extracted cochleae of P8 *Ikzf2*^{fl/fl};*Gfi1*-Cre mice.

Results: Both *Ikzf2* cKO mouse lines demonstrated a decrease in auditory function following ABR and DPOAE testing. Histological analyses showed significant membrane swelling and loss of OHCs in *Ikzf2*^{fl/fl};*Gfi1*-Cre mice, however, no OHC loss was seen in the *Ikzf2*^{fl/fl};*prestin*-CreERT2 line. SEM analyses revealed stereocilia degeneration. Transcriptomic analyses revealed an upregulation of genes associated with neuron formation and extracellular matrix organization (*Ntn1*, *Pmp22*, *Col1a2*, *Col8a1*) in cKO OHCs. Additionally, two groups of key structural genes correlating with the stereocilia (*Strc*, *Tmem145*, *Tub*, *Homer2*) and P4 ATPases that maintain membrane phosphatidylserine asymmetry (*Atp11a*, *Atp8b1*, *Atp8a2*) were downregulated. Lastly, select transmembrane ion channels were dysregulated (*Trpm3*, *Atp6v0a4* – up; *Kcnma1*, *Cacna1c* – down).

Conclusions: Our data reveal that Helios is critical not only for OHC maturation but also for maintaining OHC function. Our omics analysis illuminates novel pathways by which Helios maintains OHC homeostasis, including the regulation of phospholipid bilayer asymmetry and

appropriate transmembrane ion channel levels, with concomitant repression of OHCs from taking on a neuronal state.

T64. Long-Read Sequencing for Detection of STRC Variants

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Background: Although variants in STRC are the second most common cause of genetic hearing loss, the genomic architecture of the STRC region on 15q15.3 can make their identification challenging. As a result of a tandem duplication event, STRC has a nearby highly homologous pseudogene, STRCP1, making copy number variants (CNVs) the most frequent type of STRC genomic change. High homology complicates read mapping with short read sequencing due to misalignment. Consequently, single nucleotide variants (SNVs) in STRC can be incorrectly identified or missed due to mapping ambiguities. Manual identification and orthogonal validation methods are required for confirmation of STRC variants. In this study, we tested long-read sequencing (LRS) as a complementary technology for variant detection.

Methods: LRS with adaptive sampling using Oxford Nanopore Technologies (ONT) was done on several patients predicted to have complex STRC SNVs based on short read sequencing (OtoSCOPE®). LRS alignment and variant calling were done with an ONT-based bioinformatics pipeline.

Results: Using LRS, we could identify whether variants originated in the gene or pseudogene. Variants identified manually could also be detected using the ONT pipeline. These variants were also confirmed by orthogonal testing. LRS also allowed for phase determination, which was validated by segregation analysis.

Conclusions: This study demonstrates the potential of LRS to offer improvements in variant detection pipelines in the STRC region. However, LRS is expensive and is not simple. Region-specific expertise is essential for analysis and interpretation.

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T65. Precision Phenotyping With Audiometric Data Enhances Genetic Discovery and Polygenic Risk Prediction for Sensorineural Hearing Loss

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Background: The genetic architecture underlying hearing sensitivity as a continuous trait is critical for advancing precision medicine in hearing health, yet remains poorly understood. Despite extensive genome-wide association study (GWAS) efforts, few strong genetic

determinants have been identified, and SNP heritability remains low (Schmitz et al., 2021). A key limitation is that most GWAS studies rely on diagnostic billing codes or self-reported hearing data, which are often subjective and imprecise (Trpchevska et al., 2022; Choi et al., 2019). These methods fail to capture the full complexity of the genetic landscape underlying hearing sensitivity. We hypothesize that precision phenotyping using pure-tone averages (PTAs) derived from a large set of audiometric data exhibits superior sensitivity for detecting genetic variants associated with sensorineural hearing loss (SNHL) compared to diagnostic coding approaches. By treating hearing sensitivity as a continuous measure, this approach has the potential to enable clinically meaningful polygenic risk scores (PRS) for hearing loss prediction and risk stratification, advancing precision medicine in hearing health.

Methods: Whole genome sequencing (WGS) data from BioVU, the Vanderbilt DNA biorepository, was utilized to conduct two separate GWASs, focusing on the largest patient population - those of European ancestry. For the diagnostic code control GWAS, cases (N = 10,611) and controls (N = 52,634) were defined based on PheKB (Wei-Qi and Denny, n.d.), utilizing primarily ICD9/ICD10 diagnosis and procedure codes related to sensorineural hearing loss. For the precision phenotyping GWAS, hearing status (N = 17,326) was defined using audiograms, with the three-frequency PTA of the better hearing ear coded as a continuous variable. GWAS analyses used ridge regression in REGENIE, with functional annotation of suggestive loci. PRS were generated from summary statistics and tested in the NIH's All of Us.

Results: The PTA-based GWAS identified three genome-wide significant loci ($\lambda_{GC} = 1.038$; $h^2 = 0.086$, $SE = 0.026$), while the ICD-based GWAS identified none ($\lambda_{GC} = 1.049$; $h^2 = 0.029$, $SE = 0.008$). Genetic correlation with a hearing loss meta-analysis was higher for PTA-based GWAS ($r_g = 0.67$, $SE = 0.11$, $p = 5.2 \times 10^{-9}$) than ICD-based GWAS ($r_g = 0.38$, $SE = 0.086$, $p = 1.0 \times 10^{-5}$). PTA-derived PRS significantly predicted hearing loss ($OR = 1.089$, $95\% \text{ CI} = 1.066\text{--}1.113$, $p = 4.9 \times 10^{-15}$), whereas ICD-derived scores showed no significant associations.

Conclusions: These results demonstrate that precision phenotyping using audiometric PTA measures improves detection of genetic variants underlying sensorineural hearing loss compared to traditional diagnostic code-based approaches. The higher heritability, genome-wide significant loci, and stronger predictive performance of PTA-derived PRS suggest that leveraging quantitative hearing measures can more accurately capture the genetic architecture of hearing sensitivity. This approach enables more robust risk stratification and lays the foundation for precision medicine-based strategies in hearing health.

T66. Towards Precision Medicine in Hearing Loss: Genetic Diagnosis and Functional Studies in Zebrafish

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Background: Hearing loss (HL) is a highly prevalent condition, affecting nearly 10% of the global population, and genetic factors account for more than half of the cases. Congenital HL occurs in approximately 1 in every 500–1000 births, predominantly as non-syndromic forms (70%), usually with autosomal recessive inheritance (80%). While GJB2 and GJB6 variants represent the most frequent causes, more than 100 genes have been implicated in HL. Whole-exome sequencing (WES) has become a valuable diagnostic approach, allowing simultaneous interrogation of all candidate genes. Nonetheless, the interpretation of novel missense variants remains a major challenge for establishing robust genotype–phenotype correlations. In this study, we investigated the genetic etiology of HL in an Argentine cohort and functionally evaluated novel variants.

Methods: An initial genetic screening of 1,200 HL patients was performed using Sanger sequencing and GAP-PCR for GJB2 and GJB6 genes. In a subset of 100 unresolved cases with moderate HL, STRC deletions were tested using multiplex ligation-dependent probe amplification. Subsequently, WES was applied to 100 families (82 non-syndromic and 18 with additional clinical features, presumably syndromic). In one non-syndromic family, we identified a novel MYO6 missense variant, which was further characterized by AlphaFold2 protein modelling, followed by in vivo functional validation through zebrafish knockdown phenotype rescue assay.

Results: The GJB2/GJB6 analysis yielded a diagnostic rate of 15.5% in sporadic cases and 36% in familial cases. Screening for STRC deletions accounted for 6% of moderate HL cases. WES provided a molecular diagnosis in 45% of families, with nearly half of the pathogenic variants being novel. Among these, a MYO6 variant segregating with postlingual HL was identified. Structural modeling predicted that this variant markedly altered the protein’s surface electrostatic potential, suggesting a disruptive effect on its function. Functional validation in zebrafish experiments provided further support, as mutant mRNA failed to rescue the knockdown phenotype, confirming its pathogenicity.

Conclusions: This work highlights the power of combining comprehensive genetic testing with in-silico modeling and zebrafish functional assays to strengthen variant interpretation. The diagnostic algorithm applied in this cohort proved highly effective and supports the integration of preclinical models into routine pipelines for hereditary HL, ultimately advancing precision medicine and therapeutic perspectives in the field.

T67. A Mouse Model for TMPRSS3 DFNB8 Hearing Loss Demonstrates Progressive Hearing Loss

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Background: Pathogenic variants in the type II transmembrane serine protease 3 (TMPRSS3) gene are a common cause of autosomal recessive hearing loss in humans and can either lead to early onset severe to profound sensorineural hearing loss (DFNB10) or postlingual onset progressive sensorineural hearing loss (DFNB8). We recently showed that DFNB10 hearing loss is due to aberrant endocochlear potential leading to early hair cell death. Due to the different phenotype, we hypothesized that DFNB8 hearing loss is caused by a different mechanism than DFNB10 hearing loss. A prior mouse model of DFNB8 hearing loss (Du 2023) demonstrated onset of hearing loss at greater than one year, making experimental evaluation difficult. We sought to create a new mouse model that more closely mimics the phenotype of postlingual hearing loss seen in humans.

Methods: We identified a patient with a DFNB8 hearing loss due to compound heterozygous variants in TMPRSS3 p.His70ThrfsTer;p.A426T. We used CRISPR/Cas9 to perform embryonic gene editing directly in a C3HeB/FeJ background to induce an p.A447T mutation (equivalent to human p.A426T). This mouse was crossed a Tmprss3Y260X mouse, which had been previously backcrossed to C3HeB/FeJ to create a Tmprss3Y260X/A447T line to recapitulate the genotype of our patient. Auditory brainstem response, otoacoustic emissions, and vestibular evoked potentials (VsEP) were used to assess auditory and vestibular function. Hair cell and spiral ganglion counts were evaluated through immunofluorescence.

Results: While Tmprss3Y260X/Y260X mice show early profound hearing loss and hair cell death associated with aberrant early rise in endocochlear potential, Tmprss3Y260X/A447T mice have typical EP development and normal hair cell development, auditory brainstem response (ABR) hearing threshold, inner and outer hair cell density, and type I and II spiral ganglion neuron count through 6 months of age. After this point, ABR and OAE thresholds increase for Tmprss3Y260X/A447T mice in comparison to controls. Tmprss3Y260X/A447T mice show no inner or outer hair cell loss up to one year of age with later time points pending. Assessment of spiral ganglion neuron viability is also ongoing.

Conclusions: This new mouse model of progressive (DFNB8) TMPRSS3 hearing loss will be valuable for characterization of the mechanism of this relatively common form of hearing loss.

T68. The Contribution of Common Variants in Deafness-Associated Genes to Age-Related Hearing Loss

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Background: Age-related hearing loss (ARHL) is a broadly impacting complex disease, with both environmental and genetic factors implicated in its pathophysiology. To identify potential common genetic contributions, we tested the hypothesis that select common variants in deafness-associated genes are deleterious over a lifetime and contribute to ARHL.

Methods: Missense variants with a minor allele frequency $\geq 5\%$ in the non-Finnish European population were identified in three deafness-associated genes (CDH23, TRIOBP, and WHRN) selected based on prior studies. These variants were then rank-ordered by Combined Annotation

Dependent Depletion (CADD) score. Top-ranked variants for each gene were tested for possible association with hearing loss by examining hearing aid and cochlear implant use among All of Us Research Program participants. The relationship between variant genotype and these clinical indicators of hearing loss was assessed using logistic regression.

Results: Six of the prioritized common variants were found to be significantly associated with incidence of hearing aids or cochlear implants among All of Us participants with predicted European ancestry ($n = 234,353$). These comprised three linked CDH23 variants, two linked TRIOBP variants, and a single WHRN variant. The most strongly associated variant for CDH23 was rs4747195 ($\text{Padj} = 0.0042$, $\text{OR} = 1.18$, 95% CI 1.07–1.31), for TRIOBP was rs5756795 ($\text{Padj} = 6.4\text{e-}07$, $\text{OR} = 1.24$, 95% CI 1.15–1.34), and for WHRN was rs12339210 ($\text{Padj} = 0.017$, $\text{OR} = 1.28$, 95% CI 1.05–1.55). Analysis of cross-gene variant combinations revealed no significant departures from additivity.

Conclusions: Select common missense variants in genes implicated in monogenic hearing loss are associated with reduced auditory acuity in older adults within the United States. The impact of the variants individually is modest but cumulative across genes. These findings of common, independent genetic contributors to ARHL underscore its complexity.

T69. Development of hiPSC-Derived Inner Ear Organoids Harboring GJB2 Mutation to Model Genetic Inner Ear Hearing Loss

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Background: Hearing loss is the most prevalent sensory disorder worldwide, affecting approximately 5% of the population. Current treatment options, such as hearing aids and cochlear implants, alleviate symptoms but do not offer a permanent cure. For genetic forms of hearing loss, gene therapy holds promise as a definitive treatment. In Europe, the most common cause of autosomal recessive deafness is mutations in the GJB2 gene, which encodes Connexin 26 (Cx26), a critical component of gap junctions involved in ion transport between inner ear cells. Disruption of this ion exchange due to GJB2 mutations likely impairs auditory signal propagation, leading to hearing loss.

Methods: To investigate the cellular effects of GJB2 mutations, we employed a human inner ear organoid model derived from human induced pluripotent stem cells (hiPSCs). Using CRISPR/Cas9 gene editing, we introduced the c.35delG mutation into a healthy hiPSC line to generate a GJB2-c.35delG mutant line. Both mutant and control hiPSCs were differentiated into inner ear organoids (IEOs). Immunohistochemical analysis was performed to assess Cx26 expression in the resulting organoids.

Results: Immunohistochemical staining revealed the absence of Cx26 protein in the GJB2-c.35delG IEOs, whereas Cx26 was clearly present in the supporting cells of control organoids.

This confirms that the c.35delG mutation disrupts Cx26 expression in the inner ear organoid model.

Conclusions: The GJB2-c.35delG hiPSC-derived inner ear organoids provide a valuable human model for studying the cellular consequences of the most common GJB2 mutation associated with autosomal dominant recessive hearing loss. This model can be used for testing gene therapy approaches aimed at restoring Cx26 expression e.g. through CRISPR/Cas9-mediated gene correction, potentially paving the way for curative treatments for GJB2-related deafness.

T70. Morphological Heterogeneity Within and Between Inner and Outer Hair-Cell Bundles

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Background: Hair cells use their sensory hair bundle to transduce sound-induced forces into receptor currents. Receptor currents beget receptor potentials, which drive synaptic release by inner hair cells and cochlear amplification by outer hair cells. Inner-hair-cell bundles (IHBs) and outer-hair-cell bundles (OHBs) differ in morphology in different species, change morphology considerably during development, and change morphology substantially with cochlear location. These morphological differences and changes cause differences in function, but we lack comprehensive data on the morphology of individual bundles.

A bundle's morphology is defined by the heights, widths, and positions of the stereocilia within the bundle. Stereocilia of similar height form rows and stereocilia of differing height connected by tip links form columns. We measure the heights, widths, and positions of all the stereocilia within individual IHBs and OHBs in rats, at postnatal day 10 (P10), and near the 4-kHz characteristic-frequency location of the cochlea.

Methods: The mid-apical turn of isolated organs of Corti were dissected from Sprague-Dawley rats of both sexes at P10. Each sample was fixed using 4% paraformaldehyde in PBS. Samples were fluorescently labeled using phalloidin and antibodies against TRIOBP or ANKRD-24. Phalloidin labels stereocilium actin, enabling us to visualize stereocilium heights, widths, and positions. TRIOBP and ANKRD-24 are in the stereocilium rootlets. Stimulated emission depletion (STED) images were acquired on a STELLARIS 8 Tau-STED microscope. Images were analyzed using Imaris and Mathematica to extract the heights, widths, and positions of stereocilia within each bundle.

Results: We find considerable heterogeneity within individual bundles and between bundles. The central column of stereocilia is defined to be the column best aligned with the bundle's notch (the notch is the former location of the kinocilium that retracted during development). Bundles are asymmetric – row shapes and the number of stereocilia differ between opposite sides of the central column. Stereocilium heights and widths vary considerably within each row and tend to be smallest at the bundles' edges. In both IHBs and OHBs, stereocilium columns are far from parallel, which, according to prior work, implies heterogeneity in stereocilium deflections and channel currents within individual bundles. Rootlet lengths are correlated with stereocilium lengths in IHBs and OHBs.

Even though IHBs are from the same species, age, and cochlear location, there is considerable heterogeneity between IHBs. IHBs differ in row shape, stereocilium number, heights, and widths. We find similar heterogeneity in the population of OHBs we examined. We use simple mathematical models to calculate how heterogeneity in morphology causes heterogeneity in bundle mechanics.

Conclusions: We determine the complete morphologies of individual IHBs and OHBs for the first time. We find substantial heterogeneity within and between bundles and calculate some of the functional consequences of this heterogeneity.

T71. GIPC3 is an Organizer of MYO6-Based Transport and Endocytosis of Stereocilia Membrane Proteins in Mammalian Auditory Hair Cells

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Background: GAIP-interacting protein, C-terminus 3 (GIPC3) is a multifunctional adaptor protein essential for integrity of cochlear hair cells and hearing. Over a dozen mutations in GIPC3 cause non-syndromic hearing loss in humans; however, the exact molecular functions of GIPC3 in the auditory hair cells are still unknown. The GIPC protein family are pleiotropic adaptor proteins linking myosin motors, classically myosin VI (MYO6), with cargo to carry out a wide range of cellular processes. In cochlear hair cells, both GIPC3 and MYO6 are enriched around the cuticular plate in the pericuticular necklace (Hasson, 1997, Chatterjee et. al., 2023), the site of vesicle trafficking and endocytosis (Kachar et. al., 1997, Griesinger et. al., 2004). It was suggested that apex-to-base vesicle transport inside hair cells is MYO6-dependent (Harasztosi et. al., 2020). While the role of GIPC3 in development of cuticular plate was recently demonstrated (Chatterjee et. al., 2023), this study explores whether GIPC3 may have additional role in vesicular trafficking and endocytosis in mammalian auditory hair cells.

Methods: We couple in vitro biophysical and biochemical assays, including Hydrogen Deuterium Exchange Mass Spectrometry (HDX-MS), with crystallography to determine the structural mechanism of GIPC3 activation and its binding to MYO6. We then generated a mouse model lacking GIPC3 (Gipc3^{-/-}) along with GIPC3 polyclonal antibodies. Immunofluorescent labeling and confocal microscopy were used to determine localization of: i) GIPC3, ii) potential GIPC3 interacting partners predicted by AlphaFold3 and iii) endocytic markers in the auditory hair cells. Focused-ion beam scanning electron microscopy (FIB-SEM) was used to investigate intracellular vesicular trafficking. Finally, imaging of FM3-25 dye uptake with or without the drug inhibiting MYO6 was used to study endocytosis in live hair cells in real time.

Results: GIPC3 is normally autoinhibited by its C-terminus. This autoinhibition is released upon binding to cargo, allowing GIPC3 dimerization and interaction with MYO6. We determined the crystal structure of the GIPC3-MYO6 interacting interface to 2.5 Å. In auditory hair cells of wildtype mice, GIPC3 localizes to the cuticular plate and to regions in the pericuticular necklace, similar to MYO6. GIPC3 labeling disappeared in Gipc3^{-/-} hair cells, along with disrupted MYO6 localization and localization of some known stereocilia proteins. FIB-SEM revealed accumulation of vesicles and disrupted Golgi bodies near the cuticular plate in these cells, suggesting dysregulated vesicle trafficking. Accumulation of early endosomal marker APPL2 at

the hair cell surface suggested that at least some population of the accumulated vesicles are from disrupted endocytosis. Indeed, endocytosis of FM3-25 dye was disrupted in *Gipc3*^{-/-} hair cells, and this phenomenon was recapitulated in wildtype hair cells by applying a MYO6 inhibitor.

Conclusions: Our study defines a novel structural mechanism by which GIPC3 mediates MYO6-dependent trafficking and endocytosis in mammalian auditory hair cells.

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T72. Understanding High Frequency Vulnerability in Age-Related Hearing Loss

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Background: Age-related hearing loss (ARHL) usually starts from higher frequencies, but the underlying mechanism behind this selective vulnerability remains unclear. *Cdherin-23* (*Cdh23*) is one of the genes involved in ARHL in humans, and mouse models harbouring mutations in *Cdh23* (*Cdh23*-753A) show ARHL that starts from higher frequencies. As a major component for mechanoelectrical transduction, *Cdh23* forms the upper tip-link segment between the stereocilia—actin-based microvilli-like structures projecting from the apex of the hair cells. This structure is required for the opening of mechanoelectrical channels to transduce acoustic information into receptor potentials and drive action potentials in the auditory afferent fibres. However, it is unclear how *Cdh23*-753A affects the physiology of hair cells between higher and lower frequency regions.

Methods: To investigate the role of *Cdh23* in ARHL, hearing function and hair cell physiology were compared between C57BL/6N (6N) and C57BL/6NTac*Cdh23*⁺ (6N-Repaired) mice. The 6N mice carry the hypomorphic *Cdh23*-753A mutation, which was corrected in the coisogenic 6N-Repaired mice using genome editing. I used a variety of techniques to assess the differences between the two mouse lines. Hearing function was measured using auditory brainstem responses (ABRs). The stereociliary bundles were investigated using transmission electron microscopy (TEM). Patch-clamp electrophysiology was used to investigate physiological properties of both lower (9-12 kHz)- and higher (36-42 KHz)-frequency hair cells in postnatal day 14 (P14) mice. This technique was used to analyse basolateral and mechanoelectrical transducer (MET) currents.

Results: Hearing loss in 6N mice began at a higher frequency of 42 kHz at 1 month, and progressed to complete deafness in the 36-42 kHz region by 3 months of age. In contrast, 6N-Repaired mice maintained a lower hearing threshold. To determine whether the *Cdh23*-753A mutation causes hearing loss by affecting MET apparatus, MET currents were recorded in P14 mice, before the onset of deafness. 6N mice showed larger MET currents at higher frequencies, but not at lower frequency when compared to 6N-Repaired mice. However, the *Cdh23*-753A mutation did not affect the open probability or fast adaptation of the MET channels.

Conclusions: Our findings show that the *Cdh23*-753A mutation affects the size of the MET current in ARHL 6N mice. This effect varies across different frequency regions, and causes larger MET currents in animals carrying the mutation. Future research is needed to pinpoint the

molecular mechanisms by which changes in the MET current lead to hair cell degeneration in ARHL.

T73. An Anatomical Map of Squid Lateral Lines and Neuronal Connectivity

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Background: Survival of aquatic animals requires sensing water movement to create an internal representation of the external world. In both aquatic vertebrates and cephalopods, sensory cells on the surface of the animal detect water motion relative to the animal's body with mechanosensitive hair bundles. The lateral lines of cephalopods, also known as lateral line analogs, differ from those of fishes in the cytoskeletal composition and shape of the hair bundles and the anatomical position of the lateral line receptors. Fish have lateral lines on the head and arrayed across the body, whereas squid lateral lines have only been described in detail on the head and arms. Thus, it is an enigma how the rest of the animal's body detects local water movements. We asked what anatomical features exist on the mantle of squid that may allow them to sense water motion.

Methods: Using light and electron microscopy, we examined whole, free-swimming squid paralarvae and uncovered a complex organization of lateral lines varying in hair bundle density, anatomical position, and length.

Results: Across the exposed surface of the whole animal, hair bundle arrays are organized into discrete dense lines or broad fields, unlike what has been noted in fish or other cephalopods before. The mantle bears stereotyped arrays of hair bundles that we term mantle lateral lines. The mantle lateral lines contrast with those of the head in the substantial differences in the lengths of the hair bundles. In addition, we have mapped the connectivity of the hair bundle-bearing cells and show the locations of their central projections.

Conclusions: Our results demonstrate that the whole squid's surface is elaborately ornamented with varied lateral lines.

T74. Dissecting the Functional Connectome of the Lateral-Line System in Zebrafish

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Background: Fish and amphibia depend on their lateral line system to detect water movements around their body, which facilitates several important behaviors. The water flow information is detected by hair cells organized in clusters called neuromasts. Each neuromast transmits this information to 4-6 afferent neurons for further deciphering in the brain. Our previous work demonstrated that one thirds of hair cells in each neuromast are synaptically silent. Importantly, silent hair cells act as a reserve, and can be recruited when the active cells are damaged.

Currently it is unclear how the activities of the recruited silent synapses are reflected in the downstream circuitry.

Methods: For our analyses we have used GCaMP-based calcium imaging to examine activity in lateral line neuromasts and in all somas of the downstream afferent ganglia. We used a custom-built light-sheet microscope to perform functional in vivo imaging in all afferent neurons while stimulating a single neuromast with water jet or using optogenetic approaches. This approach enabled us to create a functional connectivity map between the afferent neurons and the hair cells in every neuromast.

Results: Our light-sheet imaging revealed that only 2 of the 4-6 afferent neurons that are anatomically connected with neuromast hair cells are activated in response to water jet stimulation of neuromasts. Further, we have performed hair cell ablation experiments that indicate a single hair cell is sufficient to activate the same portion of afferent neurons. The presence of silent afferents suggest there are also reserves at the level of afferent neurons and that the hair cell-afferent neuron connectome is very robust. We next seek to verify that silent afferents make synapses with hair cells and whether these silent afferents can be recruited after synaptic damage to active afferents. We are also using optogenetic tools to investigate how the afferent-hair cell circuit responds when silent hair cells are stimulated.

Conclusions: This research helps us understand how a mechanosensory system functions in vivo. We found that the lateral line system functions with many silent connections. To further determine the roles of these silent connections may provide valuable insight on how sensory information is encoded in mammals.

T75. The Effect of TMC1 Deafness Mutations on Cochlear Hair Cell Loss

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Background: Transmembrane channel-like protein 1 (TMC1) is an essential component of the hair cell mechanoelectrical transduction (MET) complex in inner ear, which allows us to perceive sound and balance. TMC1 has been identified as the pore-forming subunit of the MET complex and to be involved in hair cell membrane homeostasis. Auditory hair cells from mice carrying TMC1 deafness-causing mutations show signs of degeneration at different postnatal development stages, therefore, causing hearing loss. However, the molecular mechanisms of TMC1 mutations in hair cell degeneration remain unclear. Here we investigate the impact of different mutations in TMC1 on hair cell degeneration and dysregulation of membrane homeostasis.

Methods: We performed morphological and quantitative analysis of the spatial and temporal cochlear hair cell degeneration in mice harboring different TMC1 mutations. Hair cell loss was quantified via myosin7a and phalloidin counterstaining. Dysregulation of hair cell membrane homeostasis was characterized by phosphatidylserine (PS) externalization and visualized with annexin V staining.

Results: In mice carrying two copies of dominant TMC1 pore region mutation D569N, we observed hair cell degeneration in all hair cells at postnatal day 15 (P15) with increased hair cell degeneration rate from apex to base. Homozygous mice carrying the recessive mutation D528N,

which is also located at the ion conduction pore of TMC1, start to show signs of outer hair cell (OHC) degeneration at P30 and worsen from base to apex, while inner hair cell (IHC) loss occurs at P15 and progresses from apex to base. Interestingly, hair cells from mice carrying the TMC1 W554L mutation located outside the pore region, maintain normal morphology through early adulthood with only signs of mild OHC degeneration at the basal region at P30. Constitutive PS externalization was observed during hair cell degeneration in TMC1 pore mutant mice, while mice carrying the TMC1 W554L mutation showed reduced PS externalization.

Conclusions: Our data show hair cell degeneration is facilitated by TMC1 mutations that alter the MET channel properties and dysregulate hair cell membrane homeostasis. Our results suggest that TMC1 could play an essential role in hair cell survival by maintaining plasma membrane asymmetry, a novel insight that could have significant implications for our understanding of hearing loss and potential therapeutic interventions.

T76. Lipid Flippase ATP8B1 and Its Chaperone TMEM30B in Hair Cell Function, Degeneration, and AAV-Based Gene Therapy Approaches

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Background: The cochlea of the inner ear contains sensory hair cells that convert sound vibrations into electrical signals. Although proteins of the mechanotransduction (MET) complex have been extensively studied, the role of membrane lipid composition in maintaining hair cell integrity and MET function remains less clear. Ototoxic insults such as loud noise and aminoglycosides disrupt lipid homeostasis within the hair bundle, but the underlying mechanisms and their impact on hair cell function are not well understood. Recent work has revealed that the MET channel component TMC1 itself acts as a lipid scramblase, actively disrupting phospholipid asymmetry in stereocilia membranes. This discovery highlights the necessity for a counterbalancing flippase activity in hair bundles to restore and maintain membrane asymmetry, without which hair cells might degenerate. The phospholipid flippase ATP8B1 and its chaperone TMEM30B help preserve plasma membrane asymmetry by restricting phosphatidylserine (PS) to the inner leaflet. Our preliminary data show that ATP8B1 and TMEM30B knockout (KO) mice display elevated auditory brainstem response (ABR) thresholds, PS externalization, and rapid hair cell degeneration, underscoring the essential role of stereocilia flippase function for auditory function. To advance clinical translation, we identified TMEM30B as an attractive candidate for AAV-mediated gene therapy, given its single-exon structure and late-onset expression, with expression peaking around murine hearing onset.

Methods: We generated HA-tagged ATP8B1 and TMEM30B mouse models to assess protein localization by confocal microscopy. Knockout mice were evaluated using auditory brainstem response (ABR) testing and Annexin V labeling to assess membrane homeostasis. Hair bundle morphology was examined by scanning electron microscopy (SEM). We engineered an AAV vector carrying the full TMEM30B gene, including its endogenous promoter and 5'/3' UTRs. The construct will be delivered to the cochlea of TMEM30B knockout mice via posterior semicircular canal fenestration. The efficacy of AAV.TMEM30B treatment will be evaluated by ABR and immunohistochemistry (IHC).

Results: Using HA-tagged mouse models, we found that ATP8B1 and TMEM30B are enriched in hair bundles after the onset of mechanotransduction and show a rapid increase in expression following the onset of hearing, consistent with qPCR and RNA-seq data. Knockout mice exhibited altered hair bundle morphology, significant hearing loss, and hair cell death preceded by PS externalization. We anticipate that AAV-TMEM30B delivery in TMEM30B knockout mice will result in successful hair cell expression and partial preservation of ABR thresholds and hair cell survival.

Conclusions: Our findings identify ATP8B1 and TMEM30B as essential regulators of lipid asymmetry in sensory hair cells and establish TMEM30B as a novel deafness gene. Furthermore, AAV-mediated TMEM30B delivery is expected to demonstrate therapeutic potential by restoring membrane homeostasis and preserving auditory function in TMEM30B-deficient mice. These studies provide an early foundation for the development of gene therapy approaches targeting lipid flippase related hearing loss.

T77. Manipulation of Ca²⁺-Dependent PCDH15 Elasticity Through in Silico-Informed Mutant Designs

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Background: Hair cells are inner ear sensory cells that transduce sound and acceleration into electrical signals through movement of hair-like stereocilia. Apically located stereocilia are arranged in a staircase fashion and tethered together by tip link protein complexes. With stereocilia movement, tip-link proteins are taut under tension, pulling upon and opening the mechanoelectrical transduction (MET) ion channel complex. A tip link is a heterodimer of two homodimers, protocadherin 15 (PCDH15) and cadherin 23 (CDH23). PCDH15 is a long protein with 11 extracellular cadherin (EC) repeats, one membrane proximal domain, and one transmembrane segment. Interestingly, PCDH15 has elastic properties that may contribute to the mechanical sensitivity of the MET channels. The Ca²⁺ occupancy at specific EC linkers contributes to PCDH15's stiffness. To investigate whether EC linker Ca²⁺ occupancy universally affects PCDH15's elasticity, we engineered four PCDH15 variants with reduced Ca²⁺-binding. These variants will undergo a battery of in silico, in vitro, and ex vivo testing to investigate the direct effects of Ca²⁺-binding at each EC linker on PCDH15's mechanical properties and MET currents.

Methods: Guided by sequence alignments of EC linkers, we proposed mutations at crucial Ca²⁺-binding glutamate and aspartate residues in the EC linker Ca²⁺-binding motifs. We utilized SWISS modelling and AlphaFold3 to predict whether the proposed mutations ablated Ca²⁺-binding. Mutations were then made in QwikMD for molecular dynamics simulations performed with the CHARMM36 force field with 150 mM KCl in water solvent at equilibrium conditions. Ca²⁺-occupancy and steady-state flexibility of the EC linkers were recorded.

Results: Four PCDH15 variants predicted to have reduced Ca²⁺-binding were generated to probe equilibrium flexibility and non-equilibrium elasticity.

For Ca²⁺ ablation mutations at canonical binding sites, short simulations and structural predictions corroborated that one of three Ca²⁺-binding sites were ablated in four variants. Two of three Ca²⁺-binding sites were retained in short simulations. One of the two retained sites showed more Ca²⁺ movement than in wild type.

Conclusions: We engineered four PCDH15 variants aimed at removing Ca²⁺-binding. Simulations show that in silico methods are powerful hypothesis-generating tools to propose mutations ablating Ca²⁺-binding and informing wet lab experiments. We intend to perform longer simulations, in vitro Fourier-transform infrared spectroscopy, and ex vivo hair cell electrophysiology to validate the Ca²⁺-occupancy at each site and to reveal the effects of Ca²⁺-binding at each PCDH15 EC linker on MET channel activity.

T78. An Auxin-Inducible Degron Strategy to Probe MYO7A Function in Hair Cell Mechanotransduction

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Background: Sensory hair cells detect sound through mechanotransduction (MET), which relies on the deflection of actin-rich stereocilia organized into hair bundles. Tip-links connect the MET complex at its lower end to the side of the next taller stereocilium. Previous work, including ours, provided evidence that the unconventional motor protein MYO7A, located at the upper tip-link density (UTLD) is essential for tip-link tension. In addition, we hypothesize that MYO7A is required for tip-link repair by acting as a transporter of tip-link components to the UTLD insertion site, and that its loss during the repair window should impair tip-link reformation. Traditional genetic models are challenging for investigating MYO7A's post-developmental role: global or isoform-specific knockouts disrupt development and may trigger compensatory mechanisms, while conditional strategies are limited by the slow turnover of MYO7A. We therefore employed the auxin-inducible degron (AID) system to achieve rapid, reversible degradation of MYO7A in hair cells. This approach allows investigation of MYO7A's post-developmental role in tip-link repair both in vivo and ex vivo.

Methods: To generate the MYO7A-AID mouse model, a degron tag was inserted at the N-terminus of the MYO7A isoforms and combined with a knock-in of the rice OsTIR1 receptor. MYO7A remains stable under normal conditions, but upon auxin administration OsTIR1 recruits the ubiquitin–proteasome machinery to target degron-tagged MYO7A for degradation. Explant cultures and mice were treated with auxin at defined concentrations and time points to quantify MYO7A depletion and recovery.

Results: Genetic integration of the AID tag in the mouse Myo7a gene did not impact hair cell development or hearing function. In explant cultures, treatment with 500 μ M auxin for 2 h reduced MYO7A levels to 25% of WT levels. Currently, we are assessing the kinetics of MYO7A depletion and recovery in vivo. Preliminary data after treatment with 300 mg/kg auxin reduced MYO7A levels significantly relative to controls 12 h post-treatment. In contrast, a partial recovery of MYO7A levels may be observed 36 h post-treatment. These findings suggest effective, reversible depletion of MYO7A using the MYO7A-C-AID model.

Conclusions: Both ex vivo and in vivo data suggest the MYO7A-C-AID model is suitable for studying the post-developmental role of MYO7A in tip-link repair. This system enables rapid

temporal control over MYO7A depletion, which overcomes limitations of traditional genetic models. Ongoing work will continue refining the model to assess the impact of MYO7A on tip-link reformation and hearing recovery following noise exposure in vivo.

T79. Development of a Triple-Reporter Hpsc-Derived Organoid Platform for Inner Ear Disease Research

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Background: Cochlear hair cells (HCs) are essential mechanosensory receptors for hearing, yet they are highly vulnerable to damage and show minimal regenerative capacity in mammals. Loss of HCs causes permanent hearing impairment, creating an urgent need for human-relevant models of auditory development and pathology. Research has relied heavily on animal experiments, which raise ethical concerns and offer limited translational value. Human induced pluripotent stem cells (hiPSCs) provide a renewable platform to overcome these limitations. By establishing lineage-specific fluorescent reporters, HC differentiation and maturation can be visualized in real time, forming a basis for mechanistic studies and therapeutic screening. Here, we aim to create a CRISPR-based triple knock-in reporter hiPSC system targeting *Atoh1*, *Pou4f3*, and *Myo7a* to enable stepwise tracking of HC lineage progression. This system will serve as a versatile organoid platform for inner ear disease studies.

Methods: hiPSCs (ATCC BYS-0103) were maintained under feeder-free conditions and confirmed for pluripotency by OCT4 and SOX2 immunostaining. A donor vector containing *Atoh1*-P2A-GFP with a PGK-driven puromycin resistance cassette flanked by loxP sites was designed. CRISPR-Cas9 ribonucleoproteins targeted the *Atoh1* locus, and nucleofection was performed using optimized parameters, followed by puromycin selection. Resistant colonies are being expanded and characterized. Donor vectors for *Pou4f3* and *Myo7a* are under design for subsequent knock-in. Differentiation toward otic lineage and organoid formation will use stepwise modulation of Wnt, FGF, SHH, and retinoic acid signaling validated in prior organoid protocols.

Results: The *Atoh1*-GFP donor vector was generated and introduced into hiPSCs, yielding puromycin-resistant colonies under analysis. Initial assessments confirm robust survival and expansion after nucleofection. *Pou4f3* and *Myo7a* constructs are under design. By the meeting, we expect to present preliminary *Atoh1*-GFP activation data marking prosensory progenitors and progress on additional reporters. Ultimately, integration of three reporters will allow visualization of sequential steps in HC lineage progression within cochlear organoids.

Conclusions: This work presents the initial establishment of a CRISPR knock-in *Atoh1* reporter hiPSC line as the first step toward a triple-reporter system targeting *Atoh1*, *Pou4f3*, and *Myo7a*. These reporters will enable stepwise monitoring of HC differentiation, providing a dynamic and translationally relevant in vitro model. Such a platform is expected to reduce reliance on animal models, accelerate mechanistic studies, and create opportunities for drug screening and regenerative strategies. Future efforts will validate functional and electrophysiological properties of reporter-positive HCs and investigate synaptic connectivity with spiral ganglion-like neurons within organoids.

T80. Implementing Tools for Bayesian Inversion to Uncover Hair Cell and Neuronal Electrical Potentials From Calcium Imaging Data

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Background: Calcium imaging offers a valuable window into voltage changes that drive synaptic transmission and spike generation - but relative to membrane voltage, the fluorescence signal is delayed and shaped by buffering and extrusion mechanisms. Generative modeling combined with Bayesian inversion provides a principled method to extract hidden electrophysiological variables from fluorescence. Previous Bayesian deconvolution approaches for neuronal calcium imaging have been successful in estimating spiking and membrane potentials in hippocampal neurons (Rahmati et al., 2016). However, their application in cells that lack spiking, such as hair cells, remains unexplored.

Methods: We developed a biophysical model of calcium dynamics for vestibular hair cells, which incorporated voltage-dependent calcium entry through CaV1.3 channels, PMCA - mediated clearance, and the kinetics of buffers. A Hill equation links the predicted calcium transients to the fluorescence signal based on the kinetics of the calcium indicator (GCaMP8). This generative model is driven by voltage input and simulates calcium traces that can be compared to experimental data. However, when only fluorescence data is available (when hair cells are not patch-clamped), inversion methods are needed to deconvolve the fluorescence signal to a voltage waveform. We are implementing a Variational Bayesian Analysis (VBA) framework to invert and retrieve hidden voltage dynamics from calcium imaging in vestibular hair cells and neurons. The VBA approach approximates the posterior of hidden states by optimizing a lower bound, offering computational efficiency and uncertainty quantification. We validated the inversion method on ground-truth voltage-clamp protocols recorded from neuronal cells by comparing the recovered voltage to the true command waveform.

Results: Our generative model faithfully reproduces fluorescence signatures recorded from vestibular hair cells across diverse voltage waveforms. Variational Bayesian inversion also recovered the hidden voltage trajectory accurately from $\Delta F/F$ data from hippocampal neurons. Performance was robust under moderate noise and parameter variability, delivering credible uncertainty bounds on the inferred trajectories. Preliminary application to experimental imaging data from neuronal systems suggests the method can distinguish spontaneous from evoked calcium dynamics.

Conclusions: This work introduces a mechanistically grounded, Bayesian deconvolution approach for interpreting calcium signals from vestibular hair cells and calyx afferents. This method improves the interpretability of calcium imaging in neurophysiology. We plan to apply the VBA toolbox to infer voltage spiking from vestibular calyx afferents and the receptor potential from vestibular hair cells. Future extensions include adapting the model to in vivo imaging stimulation protocols and generalizing to other inner-ear cell types. Our approach exemplifies how generative modeling coupled with variational Bayesian inversion offers a powerful method to extract latent hair cell voltage dynamics from Ca²⁺ activity - expanding the toolkit for hearing-related research.

T81. P2Y Receptors Mediate Inhibition of Acetylcholine Release at the Developing Efferent-Inner Hair Cell Synapse

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Background: Before the onset of hearing (postnatal day (P) 12 in mice) inner hair cells (IHCs) are transiently innervated by medial olivocochlear (MOC) efferent fibers. Acetylcholine released by these fibers activates $\alpha 9\alpha 10$ nicotinic receptors coupled to SK2 calcium-activated potassium channels, leading to inhibitory post synaptic currents (IPSCs). During this developmental period, IHCs fire spontaneous sensory-independent action potentials that are required for normal development of the auditory pathway. Several studies suggest that this spontaneous activity is driven and/or modulated by ATP released from cochlear supporting cells and that ACh released from efferent fibers would also contribute to this regulation. The main goal of this study is to investigate the role of ATP and its products of hydrolysis, such as adenosine, in modulating the MOC-IHC synapse.

Methods: IHC from Balb/c mice at P8-11 were recorded in the whole-cell patch-clamp mode while applying different agonists and antagonists of purinergic receptors. Neurotransmitter release was evoked by electrical stimulation of the MOC fibers. Fifty double-pulse (40 Hz) stimuli were made at a frequency of 1 Hz. The quantal content (m) was estimated as the ratio between the mean amplitude of evoked synaptic currents and the mean amplitude of spontaneous synaptic currents.

Results: ATP reversibly decreased m in a concentration dependent manner compared to control (1 μ M: 10%, 10 μ M: 40%; 50 μ M: 52%, 100 μ M: 52%). The general P2 antagonist, Suramin, abolished the effect of ATP. On the contrary, TNP-ATP a specific P2X antagonist, did not modify ATP-induced inhibition. Furthermore, α, β -MeATP, a specific P2X agonist, had no effect on m. Both non-hydrolyzable ATP analog ATP γ S and the specific P2Y agonist 2-MeSADP, mimicked the effect of ATP (39% and 28%, respectively). Similar effects of ATP and ATP γ S were observed during trains of stimuli. To determine which P2Y receptor mediates this phenomenon, the specific P2Y₁ antagonist MRS2500 was used but failed to block the effect of ATP. Recent studies have shown that P2Y₁₂ and P2Y₁₄ receptors are expressed in MOC terminals. To further determine the receptor subtype involved, specific P2Y₁₂ and 14 antagonists remain to be tested. In addition, adenosine decreased m in a reversible and concentration dependent manner (1 μ M: 42%, 10 μ M: 70%, 100 μ M: 72%). CGS15943, a specific P1 receptor antagonist, abolished the effect of adenosine, while NECA, a specific P1 agonist, decreased m (10 μ M: 26%).

Conclusions: Our results suggest that both ATP and adenosine inhibit ACh release at the MOC-IHC synapse through the activation of P2Y and A₁ receptors, respectively. These results suggest that ATP may have multiple roles in the developing cochlea.

T82. Evaluating Calcium Dependence of Vesicle Replenishment at Inner Hair Cell Ribbon Synapses

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Background: The cochlea must signal continuously over long periods of time, naturally selecting for an efficient mechanism to restore the capacity to respond to new stimuli. A critical point for this phenomenon is synaptic contacts, since refilling of the “readily releasable” synaptic vesicle pool to sustain synaptic transmission is a demanding but essential presynaptic process. Synapses between inner hair cells (IHCs) and spiral ganglion neurons (SGNs) in the mammalian inner ear have structural (synaptic ribbon) and functional peculiarities that presumably contribute to an efficient recovery process. In other ribbon synapses, it is shown that the recovery process could be positively modulated by the intracellular Ca²⁺ concentration. This observation was repeated in synapses from the central nervous system, showing modulation of replenishment by global [Ca²⁺] or by previous activity level.

Methods: Explants of the organ of Corti from neonatal rats were used for experiments. Simultaneous pre- and post-synaptic patch-clamp recordings at IHC-SGN ribbon synapses were performed. Intracellular Ca²⁺ concentration was measured with a fluorescent indicator (Fluo-5F) introduced through the recording electrode along with 1 or 5 mM EGTA. Fluorescence intensity and pre- and post-synaptic currents were detected with a camera and voltage-clamp, respectively.

Results: The synaptic transmission recovery time course after 1-second-long “depleting” stimuli to -20 mV was measured with a test pulse delivered at different intervals in 1 mM EGTA (linear rate of 25%/second of the original response). Especially at the shortest stimulus intervals, we found a high probability of transmission failures. Interestingly, when EPSCs were detected after short recovery intervals, their mean amplitudes did not differ from responses to control pulses or to test pulses following longer recovery intervals. Recovery was evaluated also using a higher concentration of intracellular Ca²⁺ buffering (5 mM EGTA). During the recovery intervals, 5 mM EGTA lowered the peak [Ca²⁺] transients in IHCs by approximately 10-fold, but produced no differences in recovery rate measured over seconds. Similar rates were obtained also when delivering a partially-depleting pulse of -34 mV to the IHC, which also produced smaller Ca²⁺ currents and lower [Ca²⁺] transients.

Conclusions: These results indicate that EPSC-event amplitude does not change during evoked depression of release probability or during recovery following a stimulus. Although evoked release probability depends on level of depolarization and Ca²⁺ buffering, after the first 100 ms following a depolarization, the release probability recovered with a time course that did not appear to be modulated by the global intracellular Ca²⁺ concentration. Estimates of how many vesicles are released during the 1-sec pulses suggest that the number of vesicles tethered to ribbons would be exocytosed during -20 mV pulses in either 1 mM or 5 mM EGTA, but only partially during steps to -34 mV.

T83. Characteristic Changes in Cochlear Implant-Stimulation Out-Comes Between Human and Guinea Pig Nerve Models in Guinea Pig Anatomy: A Simulation Study

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Background: Cochlear implants (CIs) are the most successful sensory prostheses. However, stimulation outcome was shown to vary between patients, although same CI devices and settings were used under comparable conditions. Animal models are often employed to understand characteristic effects of different stimulation conditions, enabling the analysis of responses of cochlear fibers and the resulting activation of brain regions. However, altering specific conditions (e.g., anatomy, electrode positions/geometries, neuronal properties) in animal models can be challenging/unfeasible. Computer models enable detailed analysis of stimulation outcomes under defined conditions. Recently, we established a computer model of a guinea pig head based on μ CT scans, focusing on anatomical features of the cochlea and the surrounding skull. Neuronal activation and electrically evoked compound action potentials (eCAPs) were evaluated considering a biophysical model of human nerve fibers, where neuron morphology was adapted to emulate guinea pig nerve fibers. In this work, the neuron model was adapted to a cochlear nerve fiber model specific to guinea pigs and results of both models were compared to test for differences regarding nerve fiber activation and neuronal responses.

Methods: An existing computer model of a guinea pig head based on segmented μ CT scans was used. A species-adapted custom electrode array with 6 contacts (MED-EL GmbH, Austria) was considered in the cochlea, for which the contact positions were chosen based on averaged electrode positions from several in vivo experiments. An eCAP recording electrode was considered at the round window to allow for verification with performed in vivo recordings. Reference electrode contacts were considered near the contralateral ear. Nerve fibers were generated starting from equidistant points along the cochlear turns, following a path through the segmented cochlear nerve volume towards the brain. In addition to the existing human neuron model previously used in our simulations, an adapted guinea pig specific neuron model was used. Monopolar stimulation was tested at different electrode contacts and resulting eCAPs were simulated.

Results: Differences in neuronal activation thresholds and resulting eCAPs between the models were analyzed and compared to recorded data. Pending simulation results, we expect nerve fiber activation thresholds and eCAPs simulated with the guinea pig specific model to be closer to previously recorded in vivo data and that characteristic differences between simulated results will be found. Based on in vivo data, we expect differences based on stimulation polarity and grounding configuration, with anodic stimulation in monopolar configuration yielding highest threshold and lowest eCAP amplitudes.

Conclusions: By considering a model specific to guinea pig neurons, more reliable analysis of simulated stimulation scenarios will be feasible. Comparison to results obtained by the previous model with adapted human cochlear nerve fibers for the guinea pig anatomical model might provide valuable insights regarding the translation of obtained results between species.

T84. Serum Biomarkers of Neurodevelopmental Delay in Children Admitted to the Neonatal Intensive Care Unit

Serum tests which can act as biomarkers are routinely recorded in neonates admitted to the NICU but those associated with NDD have not been well documented.

Aim: To identify candidate serum biomarkers for NDD in children with BHL.

Methods: Data from 92 cases at Site 1 and from 104 cases at Site 2 were extracted from clinical records. Cases with BHL (46, Site 1; 52 Site 2) were matched for gestational age, birth weight and sex with a control group of children with no hearing loss. Daily candidate serum biomarker data were collated, assessing peak, trough and first 5-day average concentrations.

The degree of NDD was categorised as normal-mild or moderate-severe. Most 2-year developmental outcome data were extracted from online/paper notes that had been entered by paediatric consultants - specialists in neurodevelopmental follow-up.

Hearing losses were recorded from the most recent audiological testing (diagnostic ABR or VRA, PTA – age-dependant) from their paediatric audiologist.

Spearman's Rank test (two-tailed) was used to assess association between concentration of candidate serum biomarkers and degree of neurodevelopmental delay at 2-years of age.

Results: At both sites children with NICU admission and BHL had a higher incidence of moderate-severe NDD than matched controls. At Site 1, 23 of 46 children with BHL had moderate-severe NDD compared to 3 controls. At Site 2, 39 of 52 children with BHL had moderate-severe NDD compared to 8 controls.

NDD was not only attributable to the hearing loss (i.e., not only speech and language delay). More children with BHL than controls had cerebral palsy (CP). At Site 1, 13 of 46 children with BHL had CP, compared to just one control. At Site 2, 22 of 52 children with BHL had CP compared two matched controls.

Moderate correlations were found between degree of NDD and serum peak conjugated bilirubin ($r_s = .52$, $p < 0.001$), average conjugated bilirubin ($r_s = .50$, $p=0.002$), minimum albumin ($r_s = .44$, $p < 0.001$, Site 1), average albumin concentrations ($r_s = .44$, $p < 0.001$) (Site 1 data). At site 2, significant correlations were recorded between degree of NDD and minimum albumin ($r_s = .31$, $p=0.018$), and minimum platelet count ($r_s = .38$, $p < 0.001$).

Conclusions: Children with BHL are at an increased risk of NDD and CP. Identifying diagnostic biomarkers for neurodevelopmental delay is crucial for timely and accurate diagnosis, early intervention and personalised treatment. Ultimately this will improve patient outcomes, reduce healthcare burdens, and advance the understanding of the biological mechanisms underlying these complex conditions.

T85. Comparing Infant and Adult Electrophysiological Responses to Brightness Changes With and Without Variations in Pitch

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Background: Our prior behavioral studies showed that infants outperform adults on brightness discrimination in the presence of random variations in pitch, and vice versa. One interpretation of this finding is that adults have learned the statistical covariation between pitch and brightness in natural sounds, which supports efficient coding but reduces performance when this expectation is

violated. To test this hypothesis, we recorded mismatch negativity (MMN) responses to brightness changes with and without random pitch variations using electroencephalography (EEG) in infants and adults. We hypothesized that MMN amplitudes in adults would be more disrupted by random pitch variations than in infants.

Methods: The study consisted of two condition – brightness changes with and without random variations in pitch. Each condition consisted of 1500 trials with the standard trials composed of bandpass-filtered harmonic complex tones with a center frequency (CF) of 1000 Hz and slopes of 24 dB/oct on either side of the CF. Two deviant tones were randomly presented on 20% of the trials, with the first deviant corresponding to a smaller brightness change (1100 Hz CF; 10% of tones) and the second deviant corresponding to a larger brightness change (1400 Hz CF; 10% of tones). In the pitch variation condition, the fundamental frequencies of the tones were randomly roved between 180 and 220 Hz in 10-Hz steps. Each 7-month-old infant was tested across two sessions, while adults were tested in a single session.

Results: In adults, MMNs were observed to both brightness changes without pitch variation, with a smaller amplitude for the smaller change. However, in the presence of random pitch variation, no MMN was observed for the smaller change and the MMN to the larger brightness change was reduced. In infants, responses were more comparable across conditions, with smaller MMNs for the small change in both contexts.

Conclusions: Adult MMNs were strongly affected by random pitch variation, whereas infant MMNs were less clearly affected and more similar across conditions. The results are consistent with the idea that perceptual coding in adults relies on the learned pitch-brightness covariation, whereas infants may not yet have learned these statistical regularities. [Work supported by NIH grant R01 DC005216.]

T86. Cortical Gain Enhancement and Spatial Release From Masking in Hard of Hearing Children

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Background: Speech perception in noise is a critical skill across the lifespan, particularly for children who must hear in noisy classrooms to learn. Although all children experience greater difficulty in noise due to central immaturities, these challenges are heightened in children who are hard of hearing (HoH). In HoH adults, prior studies have shown enhanced cortical tracking of the speech envelope relative to typically hearing adults, consistent with a central gain mechanism. Another mechanism supporting speech-in-noise perception is spatial release from masking (SRM)—the improvement in perception when target speech and distracting noise are spatially separated. Whether cortical gain enhancement and SRM are observed during development, when the auditory system is still maturing, remains unclear.

Methods: We measured speech envelope tracking in 7–18-year-old HoH children and age- and biological sex-matched TH peers using electroencephalography (EEG). To simulate classroom listening demands, participants heard naturalistic continuous speech in three conditions: (1) Quiet (target speech alone at 0° azimuth), (2) Co-located Noise (target and four-talker babble both at 0°), and (3) Separated Noise (target at 0°, babble at ±90°). Cortical tracking was

quantified using backward Temporal Response Function modeling. A neural estimate of SRM was defined as the difference in tracking between the Separated and Co-located conditions.

Results: HoH children exhibited enhanced cortical tracking in Quiet and Co-located conditions, but not in the Separated condition. Unlike TH children, the HoH group did not show evidence of SRM at the group level, although substantial individual variability was observed in both groups.

Conclusions: Our preliminary findings suggest that hard of hearing children demonstrate cortical gain enhancement under certain listening conditions but may not benefit from spatial release from masking to the same extent as their typical hearing peers. [Supported by NIH grants R00 DC016640 and T32DC005361-21]

T87. The Role of Distorted Tonotopic Coding in Shaping Listening Outcomes in Sensorineural Hearing Loss

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Background: Clinical evaluation and management of sensorineural hearing loss (SNHL) currently emphasize measurements of hearing sensitivity and restoration of speech audibility in quiet using hearing-aid amplification. Yet, even with clinically prescribed state-of-the-art hearing aids that restore audibility, patients with SNHL continue to struggle with speech understanding, particularly in cocktail-party environments. With audibility largely restored, these suprathreshold deficits are traditionally attributed to reduced frequency selectivity (i.e., broadening of the “tip” of cochlear tuning curves) associated with SNHL, and to non-peripheral, “higher” cognitive factors. However, our cross-species studies suggest an additional mechanism: SNHL can distort the fundamental tonotopicity of the cochlea, such that the temporal responses of a given cochlear section are commandeered by “off-frequency” (i.e., substantially lower-frequency) sound fluctuations, displacing the tonotopically “correct” inputs that are otherwise audible in isolation.

Methods: We performed auditory-nerve single-unit measurements in a chinchilla model of SNHL with noise-induced permanent threshold shifts. In parallel, we conducted human studies in individuals with mild or moderate SNHL, using behavioral measures, otoacoustic emissions, and electroencephalography (EEG) to characterize distorted tonotopy.

Results: Auditory-nerve recordings in chinchillas revealed that hypersensitive tuning-curve tails were the primary driver of severely degraded speech-envelope coding. This occurred through masking effects of low-frequency energy on basal responses, manifesting both as impaired representation of higher vowel formants and near-erasure of transient responses to high-frequency consonants. The effects were particularly pronounced with naturalistic stimuli such as speech, which contain intense low-frequency components alongside softer but highly informative high-frequency content. These distortions were further exacerbated in noisy backgrounds with “pink” spectral profiles (e.g., background talkers, environmental noise). In humans with mild-moderate SNHL, we similarly observed hypersensitive tuning-curve tails and large individual variations in tip-to-tail ratios. EEG measurements showed that these hypersensitive tails were linked to impaired neural tracking of speech envelopes. Notably, the degree of distorted tonotopy also predicted large individual differences in speech-in-noise outcomes that persist despite prescriptive amplification.

Conclusions: Together, these findings indicate that distorted tonotopy is a prominent contributor to suprathreshold deficits in SNHL, observable in both animal models and human listeners.

T88. Transient Unilateral Hearing Loss Induces Bilateral but Reversible Central Gain Plasticity

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Background: Unilateral hearing loss (UHL) presents a paradox: despite preserved hearing in one ear, affected individuals often experience disproportionate difficulty with speech perception in noise (SPIN). Standard audiometric assessments do not capture these deficits, suggesting abnormalities in central auditory processing contribute to these impaired outcomes. One proposed mechanism driving SPIN deficits is maladaptive central gain, in which reduced input from one ear drives a compensatory increase in the responsiveness of central auditory pathways. While such plasticity may partially compensate for reduced audibility in quiet, it can impair SPIN by indiscriminately amplifying both target and background signals. Central gain following bilateral hearing loss is well documented in both humans and animal models, but its dynamics in UHL remain poorly understood. Moreover, whether these changes reverse to baseline after hearing restoration has not been systematically examined.

Methods: To address this gap, we developed a reversible model of UHL using unilateral earplugging in Mongolian gerbils, a rodent model with human-like hearing at speech-relevant frequencies. Animals (n=16) were recorded longitudinally at baseline, during earplugging, and after earplug removal. Envelope following responses (EFRs) were measured non-invasively with stimulus parameters emphasizing cortical versus peripheral neural generators. A central gain index was derived as the ratio of cortical to peripheral EFR amplitudes, enabling assessment of level dependence, ear-specific effects, and recovery trajectories.

Results: Earplugging for 14 days produced a stable conductive hearing loss of ~40 dB that resolved upon earplug removal. Peripheral EFR amplitudes were reduced in the plugged ear and unchanged in the unplugged ear. In contrast, cortical EFRs were enhanced relative to peripheral responses, yielding a bilateral increase in central gain: approximately five-fold in the plugged ear and 2.5-fold in the unplugged ear. Central gain enhancement was level dependent, peaking at moderate intensities (40–60 dB SPL). Critically, longitudinal recordings after earplug removal revealed that central gain returned to baseline within two weeks. These findings demonstrate that restoring hearing within this time window enables compensatory gain changes (likely hindering SPIN) to normalize, informing the therapeutic window for intervention in UHL.

Conclusions: These findings demonstrate that UHL drives robust but reversible increases in central auditory gain. The bilateral and time-limited nature of these effects suggests a window of plasticity during which maladaptive gain can normalize, highlighting the value of early intervention. This gerbil model provides a tractable platform for probing neural mechanisms underlying SPIN deficits and for testing interventions to accelerate recovery of maladaptive plasticity following hearing restoration. Ongoing work examines how central gain dynamics shape neural coding of speech-relevant cues and behavioral outcomes in a tone-in-noise detection task. Together, this work has direct implications for understanding perceptual

consequences of enhanced central gain and for optimizing timing and effectiveness of clinical rehabilitation in otolaryngology.

T89. Hair Cell Mechanotransduction in Adult Animals is Crucial to Maintain Peripheral Auditory Circuitry Throughout Life Independent of Glutamatergic Signaling

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Background: Cochlear hair cells detect sound via mechanotransduction (MET) channels. Sound information is transmitted centrally via type I spiral ganglion neurons (SGNs), which innervate inner hair cells, while type II SGNs innervate outer hair cells. Loss of MET in developing hair cells prevents proper molecular specification and innervation in SGNs. However, if MET is important for the maintenance of auditory circuitry in adult animals is unknown. This is a question of fundamental importance because hair cell functional loss is implicated in genetic forms of hearing loss, noise- and age-related forms of the disease, and in tinnitus.

Methods: We established a new mouse model that allows us to inactivate MET channels at any timepoint during their lifetime. We generated mice carrying a floxed allele of *Tmie*, which encodes an essential component of the MET channel. To inactivate MET, we crossed these mice with *Gfi1-GFP-CreERT2* mice expressing tamoxifen inducible Cre specifically in hair cells. Following tamoxifen at 4 weeks of age, loss of MET and hearing was confirmed in conditional knockout (cKO) mice using FM1-43FX and auditory brainstem responses. We assessed the impact of MET loss on hair cell morphology and molecular properties using immunohistochemistry. Peripheral SGN synapses and connectivity were assessed using confocal and super resolution imaging. The roles of MET channels and glutamate transmission were further tested using floxed mice for *Tmc1* and *Vglut3*, respectively. Lastly, adeno-associated virus was used to rescue the genetic driven hearing loss.

Results: We found that cKO of *Tmie* in mature hair cells leads to rapid loss of MET and acquired hearing loss. Morphological and molecular properties of IHCs and OHCs shift in response to *Tmie* loss. Ribbon synapses quickly degrade in cKOs, with structural defects present in the remaining synapses. Stereotyped innervation patterns of type I and type II SGNs are lost concurrently with synaptic defects. cKO of *Tmc1* phenocopies the effects seen in *Tmie* cKO. Surprisingly, loss of glutamate transmission via cKO of *Vglut3* leads to only mild defects in peripheral circuitry. Despite the loss of ribbon synapses and retraction of SGN nerve fibers, rescue of *Tmie* expression partially restores hearing in MET-deficient cKO animals. Restoration of hearing function in cKO mice is accompanied by rescue of ribbon synapses.

Conclusions: We conclude that hair cell MET has a crucial function in the maintenance of functional auditory circuitry in adult animals independent of glutamatergic signaling between hair cells and SGNs. Our findings also reveal lasting plasticity in SGNs by demonstrating that even after synaptic loss and nerve retraction, functional circuitry can be restored by rescuing MET. These findings have significant impact for a variety of diseases thought to involve defects in hair cell function such as genetic-, noise- and age-related hearing loss, as well as tinnitus.

T90. Cortico-Amygdalar Plasticity is Both a Source and Solution for Hyperacusis

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Background: Noise-induced SNHL is an upstream precipitator of tinnitus and hyperacusis. Tinnitus and hyperacusis are defined by a spectrum of sensory (e.g., loudness sensitivity) and affective symptoms (e.g., sound aversion). Excess central gain in the central auditory pathway can account for the sensory dimensions of hyperacusis, but the biological substrates for the affective dimension of these sensory disorders are less clear. Here, we used a mouse model of noise-induced SNHL to investigate whether excess central gain spilling over from the auditory cortex to the lateral amygdala (LA) – a sensory gateway to the limbic system – underlies behavioral and autonomic signatures of abnormal affective sound processing. We also tested a corollary hypothesis: That dampening excess central gain in the auditory cortex via targeted inhibitory circuit potentiation would eliminate LA excess gain and behavioral manifestations of hyperacusis.

Methods: We expressed GCaMP in lateral amygdala (LA) excitatory neurons and monitored daily changes in sound-evoked calcium activity in awake, head-fixed mice before and after noise-induced high-frequency SNHL or a sham noise exposure. We also monitored changes in autonomic hyper-arousal via pupillometry and auditory aversion via Pavlovian discriminative threat conditioning. In a subset of mice, auditory cortex hyper-responsivity was sustainably dampened via a brief bout of 40-Hz optogenetic activation of parvalbumin-expressing inhibitory neurons (PVNs).

Results: In mice with normal hearing, initially robust LA neural responses and pupil dilation to novel mid-frequency noise bands habituated over a period of several days as sounds became familiar. SNHL interfered with habituation, resulting in enhanced – rather than diminished – neural responses to spared mid-frequency sounds and elevated sound-evoked pupil dilation. Compared to sham-exposed mice, which rapidly developed CS-specific freezing, SNHL mice exhibited non-associative threat learning, indicating a generalized sound aversion. Remarkably, a single 30-min bout of 40-Hz auditory cortex PVN activation substantially and sustainably reversed these deficits in SNHL mice. LA responses returned to near-baseline magnitudes. Behaviorally, treated mice recovered selective CS+ freezing that extinguished over subsequent test sessions, comparable to controls. Inhibitory potentiation effects were specific to 40-Hz activation and were not observed in control mice that expressed a stable fluorophore without the opsin.

Conclusions: These findings suggest that cortico-amygdalar hyperactivity may be the mechanistic link connecting cochlear damage with the affective dimensions of hearing disorders like hyperacusis and tinnitus. By demonstrating that 40-Hz activation of auditory cortex PVNs reverses both limbic hyperactivity and behavioral over-generalization, we reveal that sound-evoked emotional hypersensitivity reflects reversible circuit dysfunction rather than fixed maladaptive changes. This identifies targeted neuromodulation of cortical inhibitory networks as a potential therapeutic strategy for hyperacusis and tinnitus-related distress—affective symptoms that often prove more debilitating than the hearing loss itself.

T91. Development of a Mouse Model of Human Acoustic Trauma: First Insight Into the Effects of Corticoids

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Background: Acoustic trauma (AT), operationally defined in military medicine as new-onset or exacerbated audio-vestibular symptoms (tinnitus, hearing loss, vertigo) following acute sound overexposure, lacks consensus treatment guidelines. Recognized as a leading occupational hazard in military populations, AT carries substantial clinical and operational burdens. Corticosteroid therapy persists as standard care despite unproven efficacy, with frequent progression to permanent hearing loss causing profound personal disability, occupational restrictions, and substantial compensation costs. This context necessitates novel preventive and therapeutic solutions, whose development requires deeper mechanistic understanding of AT-induced auditory pathophysiology. Current clinical assessment, relying solely on pure-tone audiometry, fails to identify specific cochlear damage or track pathophysiological processes, while apparent threshold recovery may mask persistent injury as demonstrated in animal models. Given the clinical inaccessibility of human auditory tissues, we established a murine model of acoustic trauma replicating human pathology to enable analysis of cochlear histopathology and plasma proteomic changes.

Methods: To develop a murine model of acoustic trauma, CBA/JRj male mice were used. A custom-built shock tube was designed to replicate the FAMAS rifle-induced shock wave. Several firings conditions were performed to determine the minimal number required to induce a permanent threshold shift (PTS). To evaluate the impact of corticosteroid therapy on plasma biomarkers, mice were treated with methylprednisolone. Subsequent analyses included cochlear histology and plasma proteomic profiling using Luminex technology.

Results: Following five shock tube firings, an Auditory Brainstem Response (ABR) threshold elevation of approximately 50 dB was observed across 5-40 kHz. Subsequent histological analysis of the cochleae revealed a near-total loss of outer hair cells, a 50% reduction in ribbon synaptic count in inner hair cells, and a 20% decrease in inner hair cell height. Treatment with methylprednisolone had no effect on these three parameters.

Proteomic analysis revealed increased plasma concentrations of several proteins in untreated mice at both 3h and 24h post-shock. Methylprednisolone treatment of exposed mice reduced these biomarkers to levels comparable to unexposed mice at the two time points, without improving hearing threshold.

Conclusions: We established a translationally relevant murine model of acoustic trauma that reliably replicates human-like permanent threshold shifts, enabling mechanistic investigation of AT damages.

T92. Blast-Induced Hearing Loss Accelerates Alzheimer's-Related Neuropathology

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Background: Hazardous impulse noise exposures, including blasts, can cause significant inner ear dysfunction and pose a long-term risk to neurological health. A single blast event can induce two of the strongest known risk factors for Alzheimer's Disease (AD): traumatic brain injury and hearing loss. The primary shock wave injures the brain by reducing cerebral blood flow and compromising the blood-brain barrier, while simultaneously damaging the inner ear. Hearing loss is the most prevalent service-connected disability in the United States. Brain injury and hearing loss can both accelerate cognitive decline. This study aims to reveal how blast-induced hearing loss accelerates cognitive decline and AD progression, specifically through pro-inflammatory mechanisms. Using a blast model in both wild-type and genetically susceptible APOE4 mice, we will longitudinally measure functional outcomes while assessing central and peripheral pro-inflammatory expression post-trauma. These findings have the potential to reveal novel therapeutic targets to mitigate blast-related auditory dysfunction and ultimately reduce the long-term risk of AD progression.

Methods: APOE4 and wildtype mice (n=4/genotype; 19–20 weeks) were exposed to a mild (10–13 PSI) blast under anesthesia. Sham control groups (n=4/genotype) received anesthesia but were not exposed to blast. To assess hearing damage, auditory brainstem responses (ABR) were measured across a range of frequencies (2, 4, 8, 16, 24, and 32 kHz) at baseline and on days 7, 14, and 28 post-blast. At day 28 post-blast, brain and cochlear tissues were collected for histological and biochemical analysis. In addition, cerebrovascular function was assessed on day 28 using the Laser Speckle Imaging System.

Results: No blast-induced mortality was observed, and mild blast exposure did not cause disruption of the tympanic membrane. Day 7 post-blast revealed significant shifts in ABR thresholds at all frequencies tested in WT and AD blasted mice when compared to WT and AD shams (*p < 0.05). This trend was also observed at Day 28 post-blast, suggesting permanent auditory dysfunction. Blast exposure significantly increased cerebrovascular dysfunction in the AD-blast when compared to the WT-Sham (*p < 0.01). Within the blast groups, AD-Blast had a significantly higher cerebrovascular dysfunction compared to the WT-Blast (*p < 0.05) indicating an association between blast and genotype. Using the Proinflammatory V-PLEX plate from MesoScale Discovery (MSD) system, chronic inflammation was observed from cochlear tissue collected at Day 28 with significant expression of pro-inflammatory cytokines when compared to WT-Sham (IL-1 β , *p = 0.01; IL-2 *p = 0.04). In the cortex, pro-inflammatory expression of TNF- α was observed when comparing WT-Sham to AD-Blast (*p = 0.01).

Conclusions: We have established a preclinical model that effectively captures complex interactions between hearing loss induced by blast, functional damage and pro-inflammatory alterations and long-term neurocognitive loss.

T93. Investigating the Mechanisms of Hearing Loss in FSHD Using a Transgenic DUX4 Mouse Model

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Background: Facioscapulohumeral dystrophy (FSHD) is a genetic disorder caused by contraction of the D4Z4 tandem repeat array on chromosome 4, leading to de-repression of the toxic transcription factor DUX4. Clinically, FSHD is characterized by progressive muscle weakness and atrophy, most prominently affecting the facial, scapular, and upper arm muscles. Disease onset can occur anywhere from infancy to late adulthood and varies widely in severity. Sensorineural hearing loss is seen in up to 20% of FSHD patients, but the underlying mechanism is unknown.

Methods: We used iDUX4pA mice, in which a doxycycline-inducible DUX4 transgene driven by a muscle promoter is integrated into the X chromosome. Although designed for muscle-specific induction, basal (“leaky”) DUX4 expression has been detected in non-muscle tissues; therefore, mice were not induced in these experiments. All mice were backcrossed onto an FVB background. Only females were studied, as male iDUX4pA carriers were more severely affected and did not survive to hearing onset. Auditory brainstem response (ABR) testing and distortion product otoacoustic emissions (DPOEs) were performed at 6-8 weeks at 8, 16, 32, and 45.2 kHz in iDUX4pA mice and wild-type littermates. Cochlear histology was used to assess cellular correlates of dysfunction.

Results: Uninduced iDUX4pA female mice exhibited significantly elevated thresholds at 32 kHz (iDUX4pA n = 17, WT n = 9, p = 0.0004), consistent with high-frequency hearing loss. DPOEs further revealed significant outer hair cell dysfunction at the same frequency (iDUX4pA n = 4, WT n = 3, p = 0.045). Histological analysis revealed selective loss of outer hair cells, most prominently in the basal turn of the cochlea. In response to loud tone bursts, ABR wave I amplitude and latency—reflecting auditory nerve and inner hair cell synapse function—were not significantly different between transgenic and wild-type mice at 32 kHz, supporting outer hair cell dysfunction rather than synaptopathy underlying ABR threshold elevation.

Conclusions: Our data suggest that effects of basal leaky DUX4 expression at some stage in the development of the inner ear of the iDUX4pA mouse model is sufficient to cause high-frequency hearing loss through selective outer hair cell loss. Ongoing work aims to map DUX4 expression and transcriptional changes in the cochlea.

T94. Hearing Loss and Vocalization Deficits in Two Mouse Models of Alzheimer’s Disease

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Background: Alzheimer’s disease (AD) is the most common cause of dementia, affecting millions of people worldwide. In addition to memory decline, AD is also often associated with early-onset hearing loss and vocal communication impairments. While hearing loss has been identified as the most modifiable risk factor for dementia, the mechanisms linking it to AD remain poorly understood. Transgenic mouse models carrying human genes connected to familial AD can be a powerful tool to examine these associations. APP/PS1 mice capture the

deposition of extracellular amyloid plaque pathology seen in AD patients, while 3xTg-AD mice display both amyloid plaques and intracellular neurofibrillary tangles (NFTs). The present study investigated hearing thresholds and ultrasonic vocalizations (USVs), a measure of mouse social communication, across the lifespan in these widely used AD models. Hearing was assessed using behavioral and psychoacoustic techniques that engage attention, memory, and participation, providing greater sensitivity than physiological measures.

Methods: Behavioral hearing thresholds were collected daily in male and female 3xTg-AD, APP/PS1, and wild-type (WT) control mice (n = 5 per sex per strain) across the lifespan using operant conditioning and psychophysical techniques. The same mice were recorded for USV assessment at four time points (11, 24, 52, and 68 weeks of age) across three social conditions: isolation, same-sex, and opposite-sex pairs. Calls were analyzed for number, type, bandwidth, and duration. Analyses compared outcomes across age, strain, sex, and social condition.

Results: Behavioral thresholds revealed progressive, age-related hearing loss in both sexes across all strains, which was positively correlated with a reduced number and diversity of USVs. 3xTg-AD mice exhibited significantly earlier-onset hearing loss than both WT and APP/PS1 mice, suggesting a role for NFT-specific pathology or the combination of plaques and tangles in auditory and vocal communication declines. In contrast, APP/PS1 mice showed only age-related hearing loss comparable to their WT controls. USV analysis revealed age-related reductions in call number and repertoire diversity in both models, with 3xTg-AD mice showing the most severe impairments. A sex effect was observed in the 3xTg-AD model, with males exhibiting more severe hearing and vocalization decline than females.

Conclusions: Hearing loss was positively correlated with reduced vocalization output, indicating that auditory decline contributes directly to impaired social communication in AD models. Findings demonstrate that hearing and communication deficits are early, progressive features of familial AD mouse models. The 3xTg-AD line more closely recapitulates human patterns of hearing loss and vocal communication decline, underscoring the significant role of NFTs or combined plaque and tangle pathology in familial AD. These results support USVs and auditory thresholds as promising biomarkers for early AD-related cognitive impairment and highlight the need for further studies on the mechanistic links between AD pathology, auditory processing, and communication deficits.

T95. Cortical Mechanisms of Auditory Grouping in Listeners With Different Hearing Configurations

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Background: Auditory grouping is a fundamental component of communication in noisy multi-talker environments. This process relies on binding acoustic features over time to form auditory objects. For listeners with sensorineural hearing loss (SNHL), the degree of spectral degradation and the use of hearing assistive devices such as cochlear implants (CI) or hearing aids (HA) can make detecting auditory objects more difficult. Yet it remains unknown how auditory grouping is degraded in these populations, and how neural mechanisms adapt or fail to adapt under different

listening configurations. We conducted three studies to examine mechanisms underlying auditory grouping in listeners with SNHL. Study 1 explored neural and behavioral responses to auditory object detection across different listening configurations. Study 2 examined the influence of low-, high-, and broadband-frequency cues in CI users with electroacoustic stimulation. Study 3 investigated neural mechanisms of auditory object emergence and disappearance while manipulating attention in CI users.

Methods: In Study 1, twenty normal-hearing (NH) listeners, seventeen HA users, thirty-one CI users with electroacoustic stimulation (A+E), and seventeen CI users with electric-only stimulation (E-only) participated. A stochastic figure-ground (SFG) task required detection of coherent tone pips embedded in random-frequency backgrounds (1000–8000 Hz). In Study 2, forty A+E CI users performed the SFG task with objects in low-frequency (< 500 Hz), high-frequency (> 1000 Hz), and broadband (low + high) conditions. In Study 3, sixteen CI users underwent the SFG task under active (detecting object appearance or disappearance) and passive (watching a muted movie) listening conditions. Electroencephalography was recorded in all studies. Analyses focused on task accuracy, evoked responses, and oscillatory power.

Results: In Study 1, CI users performed worse than NH and HA listeners. Evoked responses were weak in HA users and absent in CI users. Alpha and beta desynchronization (ERD) was present in all groups, and individual ERD differences predicted accuracy. Study 2 showed CI users were more accurate at detecting high- and broadband-frequency objects. Evoked responses were absent across conditions, but alpha ERD was strongest in the broadband condition. Study 3 revealed stronger beta ERD for object appearance than disappearance during active listening conditions. Passive listening reduced cortical responses across change types.

Conclusions: Our findings suggest that induced alpha and beta oscillations provide robust neural markers of auditory grouping across hearing configurations, even when evoked responses are degraded or absent in CI users. Alpha oscillations index the availability of spectral information, while beta oscillations track dynamic changes in auditory object formation, particularly when attention is engaged. These results indicate that oscillatory activity may reflect compensatory neural strategies supporting auditory scene analysis despite impaired spectral resolution. Clinically, this work highlights the value of targeting auditory grouping in rehabilitation by designing training protocols or device algorithms that enhance spectral cues.

T96. Tinnitus and Non-Tinnitus Cochlear Implant Users Show Different Cortical Responses to Speech in Noise

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Background: Reports on tinnitus effects in speech-in-noise (SiN) perception have been inconsistent. Some studies show a decline in SiN perception in tinnitus, while others find no differences. A meta-analysis confirmed that tinnitus is associated with SiN deficits, attributed to limited cognitive resources and the presence of hearing loss. Both factors are also influenced by cochlear implants (CIs), which restore hearing and enhance cognitive function. However, it remains unclear whether tinnitus affects SiN perception in CI users. Based on the view that tinnitus reflects reduced neural inhibition and increased central gain, we hypothesized that CI

users with tinnitus would show poorer neural unmasking of speech in noise, specifically smaller evoked responses to target speech compared with CI users without tinnitus. To test this hypothesis, this study examined behavioral and neural responses to SiN in CI users with and without tinnitus.

Methods: Sixty CI users participated, split into two groups: 30 with tinnitus (Tin-CI) and 30 without (NoTin-CI). Groups were matched on age, CI duration, and hearing thresholds. The Tinnitus Handicap Index (THI) was collected in the Tin-CI group. While electroencephalography was recorded, participants performed the Iowa Test of Consonant Perception (ITCP), which included 120 consonant-vowel-consonant words presented in multi-talker noise at two signal-to-noise ratios (SNRs): +7 dB (low) and +15 dB (high). The noise lasted 2 seconds, with the word onset at 1 second. Participants had to identify the word from four alternatives differing in initial consonant.

Results: Task accuracy and reaction time did not differ between groups across SNRs, indicating comparable behavioral performance. Both groups also showed similar responses to noise onset, suggesting early masker encoding was unaffected by tinnitus. Group differences emerged at word onset: while both groups exhibited clear evoked responses in the high SNR condition, only the NoTin-CI group maintained robust responses in the low SNR. The Tin-CI group showed attenuated word-related responses when the speech signal was more masked. In the Tin-CI group, THI scores were negatively correlated with N1 amplitude following word onset in the low SNR, and task accuracy was positively correlated with P2 amplitude in the same condition. These results support the hypothesis that tinnitus is linked to weaker cortical encoding of speech in noise.

Conclusions: Tinnitus did not affect SiN performance when demographic and audiological factors were controlled. However, CI users with tinnitus exhibited attenuated neural responses to target speech in the low SNR condition, indicating reduced neural unmasking. The negative correlation between tinnitus severity and N1 amplitude suggests that stronger tinnitus distress is associated with weaker cortical representation of speech in noise. Clinically, patients with tinnitus may benefit from additional rehabilitative strategies, such as auditory training or cognitive therapies, to support speech perception in noisy environments, even with a CI.

T97. Associations Among Audiometric, Doppler Hydroacoustic, and Subjective Outcomes of Venous Pulsatile Tinnitus

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Background: Venous pulsatile tinnitus (PT) has received increasing attention recently. As analyses of psychophysical and neuropsychological dimensions of venous PT are lacking, this study aimed to quantitatively and qualitatively investigate the correlation among audiometric, hydroacoustic, and subjective outcomes in patients with PT.

Methods: Fifty-five venous PT patients, with or without sigmoid sinus wall anomalies (SSWAs), were subdivided into SSWAs (n = 30) and non-SSWAs (n = 25) groups. Audiometric

and hemodynamic evaluations were assessed. Questionnaires including the Tinnitus Handicap Inventory, Hospital Anxiety and Depression Scale (HADS), and Athens Insomnia Scale (AIS) were deployed to evaluate the psychological impacts of PT.

Results: Among 55 subjects, PT frequency-related pure-tone audiometry (PTA) was significantly different between ipsilesional non-PT frequency-related PTA ($p < 0.01$), ipsilateral jugular vein compression PTA ($p < 0.01$), and contralesional ear PTA ($p < 0.01$). In contrast with the pulsatility index and

flow velocity, bilateral EOET and flow volume were significantly different ($p < 0.01$). Of the 3 questionnaire types, there was a strong correlation between HADS anxiety and AIS scores ($r = 0.658$, $p < 0.01$). The duration of PT was not correlated with subjective outcomes, and there was no statistical significance found among audiometric, hemodynamic, and subjective outcomes between SSWAs and non-SSWAs groups.

Conclusions: (1) The duration of PT was irrelevant to the increase of PTA. (2) Venous PT is the perception of vascular flow sound, in which hydroacoustic characteristics can be highly independent. (3) Anxiety, depression, and sleep disorders commonly prevail among PT patients.

T98. Innovative Intraoperative Sensor Detection of Pulsatile Tinnitus From Sigmoid Sinus Wall Anomalies: Advancing Efficacy in Resurfacing Surgery

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Background: To emphasize the surgical importance of addressing dehiscence over diverticulum in resolving pulsatile tinnitus (PT) in patients with sigmoid sinus wall anomalies (SSWAs) evidenced by intraoperative microphone monitoring.

Methods: 15 participants with PT caused by SSWAs underwent sigmoid sinus wall resurfacing surgery with an intraoperative microphone sensor monitoring examination. A sterilized LAVALIER II RØDE microphone sensor was used intraoperatively. Fourier transforms were carried out to evaluate PT loudness outcome before and after sinus wall reconstruction.

Results: PT was detected in all 15 cases who underwent intraoperative sensor monitoring during the procedure and was subsequently confirmed to have resolved after sigmoid plate reconstruction.

The reconstruction of the sinus wall led to a significant average reduction in relative PT sound intensity of 6.9 ± 2.6 dB in the frequency range of 20 Hz to 1000 Hz (paired-sample t test, $p < 0.01$). PT was resolved for all individuals who participated in intraoperative sensor monitoring.

Conclusions: 1) Effective reduction of PT can be achieved by addressing all dehiscences, including those overlying the diverticulum, without the need to exclude the diverticulum. 2) Intraoperative microphone monitoring can be crucial for ensuring surgical efficacy.

T99. Therapeutic Validation of Venous Pulsatile Tinnitus and Biomaterial Applications for Temporal Bone Reconstruction Surgery Using Multi-Sensing Platforms and Coupled Computational Techniques

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Background: The application of grafts and biomaterials is a cardinal therapeutic procedure to resolve venous pulsatile tinnitus (PT) caused by temporal bone dehiscence during transtemporal reconstructive surgery. This study quantifies the vibroacoustic characteristics of PT, sources the major transmission pathway of PT, and verifies the therapeutic effect of different material applications using joint multi-sensing platforms and coupled computational fluid dynamics (CFD) techniques.

Methods: The in vivo intraoperative acoustic and vibroacoustic characteristics of intrasinus blood flow motion and dehiscent sigmoid plate of a typical venous PT patient were investigated using acoustic and displacement sensors. The acoustical, morphological, and mechanical properties of the dehiscent sigmoid plate, grafts harvested from a cadaveric head, and other biomaterials were acquired using acoustical impedance tubes, micro-CT, scanning electron microscopy, and mercury porosimetry, as appropriate. To analyze the therapeutic effect of our previous reconstructive techniques, coupled CFD simulations were performed using the acquired mechanical properties of biomaterials and patient-specific radiologic data.

Results: The peak in vivo intraoperatively gauged, peak simulated vibroacoustic and peak simulated hydroacoustic amplitude of PT prior to sigmoid plate reconstruction were 64.0, 70.4, and 72.8 dB, respectively. After the solidified gelatin sponge–bone wax repair technique, the intraoperative gauged peak amplitude of PT was reduced from 64.0 to 47.3 dB. Among three different reconstructive techniques based on CFD results, the vibroacoustic and hydroacoustic sounds were reduced to 65.9 and 68.6 dB (temporalis–cartilage technique), 63.5 and 63.1 dB (solidified gelatin sponge technique), and 42.4 and 39.2 dB (solidified gelatin sponge–bone wax technique).

Conclusions: The sensation of PT correlates with the motion and impact from venous flow, causing vibroacoustic and hydroacoustic sources that transmit via air conduction transmission pathways. The transtemporal reconstructive surgical efficacy depends on the established areal density of applied grafts and/or biomaterials, in which the total transmission loss of PT should surpass the amplitude of the measured loudness of PT.

T100. How Loud Is Venous Pulsatile Tinnitus? Computational Fluid Dynamics Simulation and Transcanal Recording Techniques

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Background: Investigations of pulsatile tinnitus (PT) caused by sigmoid sinus wall anomalies (SSWAs) using computational fluid dynamics (CFD) have recently increased in prevalence. This study incorporates coupled CFD techniques, micro-computed tomography, and scanning electron microscopy to reveal the vibroacoustic and hydroacoustic sources and displacement characteristics of the transverse-sigmoid sinus system.

Methods: The transient state coupled CFD technique was used to calculate the vibroacoustic and hydroacoustic sources. The dehiscent sigmoid plate and periosteal dura mater were then reconstructed. The displacement characteristics and acoustic results were analyzed.

Results: The displacement of the vascular wall underneath the dehiscent area was 9.6 times larger than that of the sigmoid plate and 3,617 times smaller than that of the vascular wall without the overlying osseous structures. The peak amplitude of flow-induced vibroacoustic noise was 119.3 dB at 20.2 Hz measured at the transverse sinus. Within the observed 20–1,000 Hz frequency range, the largest peak amplitude of hydroacoustic noise was 80.0 dB at 20.2 Hz located at the jugular bulb region. The simulated results conformed with the in vivo acoustic profile which the major frequency of PT falls within 1,000 Hz

Conclusions: The sigmoid plate and dura mater greatly impact vascular wall displacement, which should not be overlooked in CFD simulations. PT was found fluctuating below 1,000 Hz using the transcanal recording technique, which matches the frequency component simulated by the current CFD technique; amplitude-wise, however, the peak amplitude of in vivo pulsesynchronous somatosound measures approximately 10 dB, which is comparatively lesser than the CFD results and the subjectively perceived loudness of PT.

T101. Cortical Auditory Evoked Potential Characters in Migraine Patients With Hyperacusis

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Background: A variety of abnormal sensory perceptions are frequently associated with migraine, including several otologic symptoms. A higher incidence rate of cochlear disorders was reported in the migraine cohort compared to the non-migraine cohort. Hyperacusis, an unusual intolerance to everyday sounds, is one of the most common abnormal sensory perceptions associated with migraine. Hyperacusis often improves after migraine prophylactic treatment. We hypothesize that potential differences in central auditory pathway activity between patients with and without hyperacusis may lead to distinct treatment responses.

The aim of our study was to investigate the cortical auditory evoked potential (CAEP) in migraine patients to provide insights into the disease mechanism and further management.

Methods: The study prospectively enrolled patients with cochleovestibular symptoms, while the control group comprised healthy individuals with normal hearing and no previous history of otologic disorders or headaches. All participants underwent hearing and vestibular function tests, CAEP, and completed a series of questionnaires.

We further divided disease group into patients with or without hyperacusis for further elucidation of the role of hyperacusis in the population of cochlear migraine.

Results: We totally enrolled 20 patients (2 men and 18 women) and 31 health control (14 men and 17 women). The average age was 39.6 ± 9.1 and 32.2 ± 9.6 y, respectively. The migraine group showed significantly higher Five-item Brief Symptom Rating Scale (BSRS-5) and The Chinese version Pittsburgh Sleep Quality Index (CPSQI) scores after adjustment for age and sex, and exhibited higher average pure tone audiometry (PTA) threshold in both ears despite normal hearing. The adjusted odds ratios were 6.66 for the right ear and 7.73. The auditory brainstem response (ABR) showed significantly shorter latency of wave I, III and V at the left side in the diseased group ($p = 0.037$, < 0.001 , 0.021 , respectively). The interpeak latency between wave I-III also shortened at the left side in the diseased group ($p = 0.028$). For CAEP, only the P1-N1 interval at 4k Hz of left ear markedly shortened in the diseased group ($p = 0.036$).

The disease group comprised 10 patients with hyperacusis and 10 without. The demographic data, questionnaires, hearing, vestibular function test and CAEP showed no difference between groups. The mean uncomfortable level was lower in the hyperacusis group ($p = 0.026$).

Conclusions: Migraine patients with cochleovestibular symptoms showed higher sleep and emotional difficulties compared to the normal population. In addition, the diseased group showed elevated PTA threshold, shorter ABR latency and shorter interpeak latencies of CAEP at high frequencies, implying increased sensitivity to sound. The CAEP between migraine patients with hyperacusis and non-hyperacusis patients showed no difference, indicating other adjusting mechanisms beyond the auditory pathway for hyperacusis.

T102. The Story of Fire and Mice – the Impact of Macrophage Subpopulations on Hearing

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Background: The immune system is known to critically regulate the central and peripheral nervous system during development, homeostasis, and repair after injury. Several recent studies on the mouse cochlea have reported diverse roles of macrophages including mediating cisplatin uptake, promoting synaptic repair after noise exposure, and participating in tissue remodeling after cochlear implantation. Using single cell RNA sequencing, we have revealed five molecularly diverse populations of macrophages within the mature mouse cochlea and further validated their presence in the human cochlea. The transcriptome of one macrophage subtype resembles that of CNS microglia, expressing classic microglia genes such as *Tmem119* and *P2ry12*.

Methods: To begin to ascertain the functions of this newly uncovered population cochlear microglia, we examined FIRE mice (*Csf1r* Δ FIRE/ Δ FIRE), which are deficient in microglia. These mice were firstly examined at P5, P14 and 6 weeks of age under homeostatic conditions. Next, FIRE mice were subjected to a noise exposure paradigm which was designed to induce a temporary threshold shift. Mice were functionally and histologically examined at one day, one week and two weeks after exposure, as were age matched noise exposed controls.

Results: At all ages examined, under homeostatic conditions, FIRE mice displayed normal cochlear anatomy and function indistinguishable from age-matched, wildtype controls. One day

after noise exposure FIRE mice had an auditory brainstem response threshold shift that recovered by two weeks, comparable to age-matched, noise-exposed control mice. Similarly, no hair cell loss was observed. Interestingly however, relative to control mice, a decrease in wave I amplitude of the auditory brainstem response was not observed in FIRE mice. Ongoing experiments will assess hair cell synapse integrity.

Conclusions: These findings suggest that subpopulations of macrophages within the cochlea may play varying roles under pathological conditions, underscoring the importance of studying macrophage heterogeneity in the cochlea to better understand the role macrophages play in hearing health and disease.

T103. The Auditory Nerve Glial Transition Zone: A Central-Peripheral Niche Vulnerable to Age-Related Glial Dysfunction

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Background: Age-related hearing loss (ARHL) is a growing public health concern, affecting two-thirds of adults over 65 years old. While ARHL is a multifactorial disorder, glial dysfunction remains an understudied contributor. The auditory nerve (AN) contains a diverse population of immune cells and myelinating glia (e.g. Schwann cells, oligodendrocytes, macrophages, and microglia). As the AN fibers cross into the cochlear modiolus, they reach a glial transition zone (GTZ), a region where myelination shifts from Schwann cells to oligodendrocytes. While such peripheral-to-central glial interfaces are recognized in other cranial nerves, the GTZ is understudied in the auditory system. This region integrates the peripheral and central microenvironments within the confined bony cochlea, positioning it as a niche for glial dysfunction in pathological conditions. We hypothesize that the GTZ is a site of enhanced glial dysfunction that contributes to age-related AN demyelination. We evaluate this in a mouse model of ARHL using RNA-sequencing, quantitative immunohistochemistry, and a whole-cochlea tissue clearing approach. The AN GTZ was also evaluated in human temporal bones.

Methods: Bulk RNA-sequencing of ANs from young (3M) and aged (2.5Y) CBA/CaJ mice was performed to assess age-related changes in inflammation and myelination. Myelination and macrophage/microglia-associated inflammation were evaluated using high-resolution imaging and quantitative immunohistochemistry. Immune cell activation was determined by macrophage/microglia morphology, volume, and surface area. Myelin volume and surface area were also examined with aging. Qualitative assessments of myelination and immune activity were performed in human temporal bones. To visualize the architecture of AN myelination and the AN GTZ, we developed a tissue-clearing protocol tailored to mouse cochlea and applied large-field light-sheet microscopy to generate 3D reconstructions of the ANs from young and aged mice.

Results: RNA-sequencing results revealed that aging led to significant increases in abnormal inflammation and myelination. Quantitative analysis of AN mid-modiolar sections showed a significant age-dependent increase in macrophage/microglia number and a two-fold increase in volume, suggesting greater macrophage/microglia activation in the aged AN. Myelin volume was significantly reduced compared to young mice. We defined the GTZ within the AN in mouse and human temporal bones, highlighting a novel site of central-peripheral glial

interactions. Macrophages/microglia contained significantly more internalized myelin debris in the peripheral and central AN with aging, while internalized healthy myelin was observed exclusively at the GTZ, suggesting selective phagocytic activity with demyelination.

Conclusions: Aging alters the AN in both mice and humans, including increased macrophage/microglia activation, decreased myelination, and disrupted immune-glial interactions specific to the GTZ. Findings suggest this previously unrecognized interface may serve as a critical site of immune-glial interactions and appears especially vulnerable to age-related demyelination and neuroinflammation. These results highlight the GTZ as a potential therapeutic target for preserving AN myelination, reducing pathologic neuroinflammation, and mitigating ARHL.

T104. The Origin of Cochlear Macrophages in Csom

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Background: Chronic suppurative otitis media (CSOM) is the most common cause of sensorineural hearing loss (SNHL) among children in the developing countries. Our group developed and validated an animal model that replicates human CSOM. In this model, which is induced by a pathogen in the middle ear, macrophages were identified as the major immune cell associated with outer hair cell (OHC) loss in the cochlea. Furthermore, OHCs are protected when macrophages are depleted and when the NLRP3 inflammasome is deleted in CSOM. These findings strongly suggest that the NLRP3 inflammasome activation in cochlear macrophages causes OHC loss in CSOM. However, the origin of elevated macrophages remains unknown.

Methods: In current study, we investigated the origin of increased cochlear macrophages in CSOM. Cx3cr1GFP-Ccr2RFP dual-reporter mice were used. The 5-ethynyl-2'-deoxyuridine (EdU) was injected intraperitoneally into the CSOM mouse model following the middle ear infection

Results: The EdU positive cells overlapped with Cx3cr1-GFP macrophages. The number of EdU-positive cells after 5 days of injection was significantly increased compared with 3 days of injection. However, only a few Ccr2-RFP positive cells were observed in the CSOM cochlea, and no EdU positive cells overlapped with Ccr2-RFP cells.

Conclusions: These data suggest that proliferation occurs in both the resident and infiltrating Cx3cr1-GFP macrophages, and that both populations contribute to hearing loss in CSOM.

T105. Single Nucleus RNA-Seq Defines the Cochlear Interferon Response in a Mouse Model of Congenital CMV Infection

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Background: Congenital CMV (cCMV) infection is the single leading cause of non-genetic hearing loss at birth. While the cause of hearing loss in cCMV infection is not currently well understood, the current evidence supports a mechanism of immune mediated cochlear damage

and suggests a primary role for interferon signaling. Nonetheless the timecourse, cellular players, and offshoot transcriptional effects of cochlear interferon signaling in cCMV infection are still not well characterized.

Methods: C57BL/6 mice were infected with murine CMV (MCMV) via intra-peritoneal injection at P0, and cochleae were collected at P3, P7, and P14 for single-nucleus RNA sequencing (snRNA-seq). The cochlear membranous labyrinth was dissected and separated into neurosensory (modiolus + organ of Corti) and lateral wall tissues. Single nuclei were isolated from tissues pooled from n = 10 mice, and snRNA-seq was performed with the 10x Genomics Chromium and Illumina NovaSeq Platforms. Age- and sex- matched MCMV+ and control tissues were paired for snRNA-seq, and each experiment was performed in triplicate. snRNA-seq data analysis was performed in R v4.4.2. Quality control, normalization, dimension reduction, and clustering were performed with the Seurat package. Doublets were removed using DoubletFinder.

Results: Over 300,000 high quality single nuclei were retained for analysis. Marker gene analysis allowed for the identification of many known cochlear cell types, including spiral ganglion neurons (SGNs); hair cells; stria vascularis basal, intermediate, and marginal cells; Reissner's membrane epithelium; and cochlear fibrocytes, macrophages, and supporting cells; among others. At P3 there was minimal interferon response in MCMV-infected neurosensory or lateral wall tissues. By P7, and until at least P14, type I (e.g., *Irgm1*, *Iigp1*, *Irgm2*) and type II interferon response genes (e.g., *Gbp3*, *Gbp7*) were significantly upregulated in a subset of cell types in the cochlea, most strikingly in endothelial cell and cochlear macrophage clusters, but also in some glial, cochlear epithelial, and otic mesenchymal cells. Gene-level expression of these interferon response genes was notably absent in hair cells and only rarely seen in SGNs. The timing of onset of the interferon transcriptional response coincided with MCMV entry into the cochlea. Weighted correlation gene network analysis (WCGNA) identified genes associated with interferon responses in MCMV infected cochlear cells. Trajectory (pseudotime) analysis, cell-cell signaling analysis, and other computational tools were utilized to further explore the effects of MCMV infection on gene expression in the mouse cochlea.

Conclusions: MCMV infection was hallmarked by a widespread interferon response in the mouse cochlea which is largely absent in SGNs, hair cells, and marginal cells and coincides with MCMV entry into the cochlea. This work has potentially important implications in our understanding of the pathogenesis of cCMV related hearing loss in humans, as well as in the development of treatments for this common form of hearing loss.

T106. Complement C3 Depletion Reduces Noise-Induced Cochlear Damage and Protects Hearing by Attenuating Chronic Cochlear Inflammation

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Background: Noise-induced hearing loss (NIHL) is characterized by pathological changes and dysfunction of the cochlea, including the loss of sensory hair cells and connected synapses. Growing evidence has revealed that the immune system plays critical roles in shaping cochlear responses to tissue damage and the clearance of damaged cells after noise injury. However, the underlying mechanisms that regulate this process remain unclear. The complement system, a

crucial part of the innate immune system, is known to respond to tissue damage and help remove cellular debris. Such functions are yet to be fully explored in the auditory system, especially in the cochlea. Our prior work revealed that complement C3 is activated and dynamically opsonizes damaged structures within the organ of Corti in CBA/CaJ mice after a 2-hour noise exposure at 112 dB SPL. However, when investigating the role of C3 activation in NIHL, the same noise injury caused similar NIHL in control and C3-deficient mice, likely due to a floor effect from the severe injury. In this study, we sought to further assess the functional impacts of C3 activation and its underlying mechanisms in the context of moderate cochlea damage induced by 106 dB SPL noise exposure.

Methods: To assess the role of C3 in NIHL, we compared auditory and cellular outcomes between wild-type C57BL/6 mice and homozygous C3 knockout mice (C3KO). Mice were exposed to an octave-band noise (8–16 kHz) at 106 dB SPL for two hours. Auditory brainstem response (ABR) thresholds were recorded at multiple post-exposure time points to evaluate hearing function. Cochleae were harvested for whole-mount and cryosection immunohistochemistry using antibodies against various cellular markers including sensory hair cells, ribbon synapses, and macrophages to probe the potential mechanisms by which C3 activation is impacting cellular pathology, cochlear inflammation, and hearing loss.

Results: We found that compared to wild-type mice, C3KO showed similar cochlea damage and hearing loss acutely within 2 weeks after noise exposure. However, progressive loss of outer hair cells and ribbon synapses as well as hearing loss were attenuated in C3KO mice at later time point. These protective effects were accompanied by a significant reduction in macrophage density and activation, along with reduced expression of other inflammatory markers, suggesting that chronic cochlear inflammation, contributed by C3 activity, exacerbates noise-induced pathology and is attenuated in the absence of C3.

Conclusions: Our findings demonstrate that complement C3 contributes to long-term cochlear damage and inflammation following noise injury. Chronic activation of C3 drives sustained cochlear inflammation, which underlies the progressive loss of hair cells, synapses, and auditory function. Targeting C3 or its downstream signaling pathways may offer long-term protection against inflammation-driven hearing loss.

T107. Characterization of Noise-Exposure–Driven Immune Alterations in a Humanized DFNA9 Mouse Model

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Background: Noise exposure is a well-established activator of cochlear inflammatory responses, yet its systemic immunological consequences remain less explored. Recent evidence has implicated cochlin, a major extracellular matrix protein of the inner ear, not only in cochlear immune regulation but also in peripheral immune responses. Mutations in the COCH gene, such as the pathogenic P51S variant causing DFNA9, provide a unique model to investigate the

interplay between genetic predisposition and noise-induced immune activation. However, the impact of noise on peripheral immunity in cochlin-mutated mice has not yet been addressed.

Methods: In the current experiment we've exposed wildtype and homozygous DFNA9(P51S/P51S) mice to 115dB of noise for two hours. Three and six days following noise exposure hearing function was determined with ABR and DPOAE measurements. Seven days following noise exposure the spleen, cochleae, cervical lymph nodes and blood was collected. High parametric flow cytometry was performed on the spleen, cervical lymph nodes and blood. Transcriptomics and proteomics are performed on the cochleae and data analysis is ongoing.

Results: Severe hearing loss was confirmed three and six days following noise exposure, with no observed recovery within this timespan. High parametric flow cytometry revealed altered T-cell responses in cervical lymph nodes of wildtype mice post-noise, while this effect was not detected in the DFNA9(P51S/P51S) mice. Additionally, DFNA9(P51S/P51S) mice showed altered T-cell responses in the spleen following noise exposure.

Conclusions: Current research reveals different noise-induced peripheral immune responses in our DFNA9(P51S/P51S) mouse model compared to wildtype mice. Future transcriptomic and proteomic analyses on the cochleae will reveal the local immune responses following noise and clarify how they correlate to the peripheral condition.

T108. Primary Neural Degeneration After Cochlear Implantation: Histological and Electrophysiological Evidence From a Mouse Model

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Background: Traditional cochlear implants (CIs) extend through most of the cochlear spiral, whereas hearing-preservation CIs avoid the cochlear apex to maintain low-frequency acoustic hearing. Nevertheless, some patients develop progressive loss of residual hearing, and a recent study revealed massive loss of apical hair cells in one such case. It remains unclear whether implantation routinely compromises apical hair cells, their synapses and/or associated neurons. To address these questions, we analyzed histology and electrophysiology at different times after CI insertion in a mouse model.

Methods: Eight-week-old CBA/CaJ mice were unilaterally implanted through the round window with three-electrode-contact custom CIs (Cochlear Ltd., Sydney, Australia). ABRs, DPOAEs, and cochlear microphonics were recorded via subcutaneous electrodes immediately before sacrifice at 4 days, 8 weeks, or 16 weeks post-implantation. Cochleae were microdissected, immunostained for CtBP2 (ribbons), GluR2 (postsynaptic patches), NF200 (auditory nerve peripheral axons), and Myosin VI/VIIa (hair cells), and imaged on a Leica Stellaris 5 confocal microscope. For each cochlea, the entire sensory epithelium was captured in low-resolution z-stacks (7.6 pixels/μm, 3 μm step) and processed in MATLAB to generate cytochleograms, and high-resolution stacks (25.6 pixels/μm, 0.3 μm step) were acquired at half-octave intervals from 5.6–64 kHz for synapse and auditory nerve analysis.

Results: CIs extended 1.6 mm from the round window, with the tip at roughly the 27 kHz location. Outer hair cell (OHC) loss began at the 27 kHz place immediately after implantation and progressed bidirectionally: the basal turn was completely denuded of OHCs by 8 weeks, while apical loss extended beyond the implant tip, leaving 46% of OHCs in the apical half at 16 weeks. In contrast, IHCs were largely preserved, with 94% and 60% remaining intact in the apical and basal halves at 16 weeks, respectively. Low-frequency ABR thresholds were elevated by 40 dB immediately after insertion, but remained stable thereafter. In contrast, wave I amplitudes declined progressively. At 4 days post-insertion, synapse loss appeared at the extreme apex and near the CI tip, spreading across the cochlear spiral thereafter. Peripheral axon degeneration lagged, with 30% afferent fiber loss versus 68% synaptic loss (both evenly distributed across cochlear spiral) by 16 weeks.

Conclusions: These data suggest that progressive OHC degeneration can occur despite a stable threshold shift after insertion of a hearing-preservation CI. In contrast, IHCs are relatively robust, showing little vulnerability to implantation-induced loss. In the apical half of the cochlea, we find clear evidence of CI-induced primary neural degeneration, with synapses between auditory nerve terminals and IHCs emerging as the most vulnerable elements. Moreover, ABR wave I amplitude tracks synapse survival faithfully even after correcting for threshold shift, underscoring its potential as a biomarker of neural integrity in the implanted ear.

T109. Insights Into the Spiral Lamina Vasculature of the Cochlea: New Features of Barrier Function in an Under-Studied Network

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Background: The capillary network of the cochlea's spiral lamina is relatively under-studied compared to the vasculature of the lateral wall. The proximity of spiral lamina vessels to the Organ of Corti and its supporting cells, however, suggests their importance in maintaining cochlear homeostasis. Our understanding of the function of this network is limited to local oxygen delivery and nutritive support. Here we investigate the features of this vascular network in disease models of vascular hearing loss with disrupted Norrin signalling, including the Ndp-knockout mouse (Ndp-KO). This model has previously been shown to have morphologically abnormal and leaky vessels in the stria 5 brainstem response thresholds observed at 2 months of age compared to wild-type mice (PMID: 35132964). The vessels of the spiral lamina have not been investigated previously, and we report a novel insight into their characteristics.

Methods: Cochleae were collected from adult wild-type and Ndp-KO C57BL6 mice at 2 months of age, one hour after intraperitoneal injection of 5mg Sulfo-NHS-LC-biotin, a vascular tracer. Paraformaldehyde fixation and decalcification was then undertaken to allow for dissection to remove the lateral wall and expose the Organ of Corti attached to the modiolus. Immunostaining, embedding in agarose gel, dehydration and tissue clearing was performed to allow for 3D imaging of the spiral lamina vasculature.

Results: Claudin-5 (a tight junction protein typically found in barrier vessels of the central nervous system) was detected abundantly in vessels throughout the spiral lamina vascular network in wild-type mice, signifying likely barrier function of these vessels. A reduction in

Claudin-5 was observed in the spiral lamina vessels of Ndp-KO mice compared to that observed in wild-type mice. Additionally, the normal intravascular retention of Sulfo-NHS-LC-biotin was disrupted in knockout mice, with significant leak of the vascular tracer into the surrounding tissue.

Conclusions: We present the novel finding that the vessels of the spiral lamina have the key hallmarks of a barrier network, which are disrupted in disease models associated with vascular hearing loss. This new insight has important implications for expanding our knowledge of cochlear homeostasis and its maintenance. Furthering our understanding of this vascular network may generate insights into the mechanisms contributing to cochlear degeneration and hair cell death in vascular disease.

T110. Quantifying Spiral Ganglion Neurons in Human Cochleae Using a Deep Learning Model: Presenting a Generic Model That is Flexible and Adaptable

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Background: Spiral ganglion neurons (SGNs) are crucial for transmitting auditory information to the brain. For decades, SGNs have been quantified in human cochleae to study various hearing loss pathologies in human temporal bone (TB) specimens. Quantification of SGNs in human TBs has traditionally required a trained researcher and has been a laborious and time-consuming process, taking many hours per cochlea depending on the neural density. This study introduces and assesses the accuracy of a deep learning model to quantify SGNs, aiming to streamline this process.

Methods: Using Dragonfly 2024.1., we developed a deep learning model to identify and quantify SGNs using digitized slides from the Massachusetts Eye and Ear Otopathology Laboratory Collection. The model was trained using six distinct human TB specimens that represent a range of ages, post-mortem times, and diagnoses. Ground truth annotations were generated by manually segmenting the nucleus and cytoplasm of each individual SGN on a cropped, mid-modiolar slide and used as the training dataset. Model performance was evaluated by applying it to 18 different cases with varied ages, diagnoses, and post-mortem times. SGNs identified by the model were compared to manual counts within seven predefined regions of Rosenthal's canal (RC). Additionally, one complete case was assessed across all digitized slides within RC. True positives, false positives (such as artifacts and Schwann cells), and false negatives (missed SGNs) were recorded. For specimens in which accuracy fell below 85%, five small regions were manually selected and edited to augment the training dataset. The model was subsequently retrained and reassessed to evaluate performance improvement.

Results: A U-Net convolutional neural network model was developed and trained with an average Dice coefficient of 0.91 on the validation set. Across the 18 analyzed cases, the model had a mean accuracy of 91.7% with individual case accuracies ranging from 82.4% to 95.0% relative to manual quantification. For the case with the lowest initial accuracy (82.4%), retraining the model using additional manually segmented areas improved accuracy to 92.4%. For the case

in which every digitized slide throughout RC was evaluated, the model performed with an overall accuracy of 92.2% in comparison to manual quantification.

Conclusions: We present a novel approach for quantifying SGNs in human temporal bone specimens using a deep learning model. This approach improves efficiency while maintaining high accuracy in comparison to manual quantification. The model performs robustly throughout the cochlea. Furthermore, the model can be retrained and adapted to cases that initially perform poorly with minimal additional effort, demonstrating its flexibility and potential to be applied to a diverse set of cases.

T111. Cochlear Histological Characterization in Aging Nonhuman Primates

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Background: Age-related hearing loss (ARHL) is a common, progressive type of inner ear pathology associated with complex patterns of degeneration to the ascending and descending auditory pathways. While loss of cochlear inner and outer hair cells and primary afferents is common, changes to the lateral and medial olivocochlear (OC) efferent neurons remain understudied. The OC systems are thought to support hearing in complex listening environments – an ability that declines with age even in the absence of hearing loss. Studying the OC systems directly in humans is difficult, making animal models essential for understanding their structure and function. Nonhuman primates are a useful model for human inner ear pathologies, due to their comparable hearing ranges and phylogenetic proximity. The purpose of this study was to examine cochlear histology in aging marmosets and macaques by assessing hair cell survival, afferent synapse counts, and olivocochlear efferent innervation along the tonotopic axis.

Methods: Temporal bones were collected from 13 marmosets between the ages of 0-12 years old (young < 9 years: n=8; old ≥ 9 years: n=5) and 19 macaques between the ages of 6-35 years old (young < 12 years: n=9; old ≥ 26 years: n=10), covering most of each species' lifespan. Cochleas were decalcified, immunolabeled, and dissected into 10-11 pieces. Specimens were labeled for hair cells (Myo7a), afferent synapses (CtBP2, GluA2 or GluR2), and cholinergic OC neurons (ChAT or SV2). Confocal z-stack images were obtained at octave-spaced positions along the cochlear spiral from 0.25-32 kHz. Hair cell survival and inner hair cell synapse counts were quantified. Maximum intensity projection images of ChAT or SV2 label in the inner and outer hair cell regions were auto-thresholded in FIJI to quantify lateral and medial OC innervation density (area per hair cell), respectively.

Results: Overall, we found minimal age-related cochlear structural changes in both marmosets and macaques. Outer and inner hair cell survival was generally > 85%, except at the highest frequencies in old macaques. Compared to younger animals, older nonhuman primates showed

modest reductions to afferent synapse counts across the frequency range. Marmosets had no age-related differences in medial OC innervation density, but showed a reduction in lateral OC innervation density at low frequencies. Efferent analyses in macaque ears are ongoing.

Conclusions: While prior studies in common laboratory models and human temporal bones have reported age-related reductions in hair cell counts, afferent synapse counts, and medial OC innervation, our findings in aging nonhuman primates reveal minimal structural changes. These results contribute to our understanding of aging in the cochlea and the utility of nonhuman primates as a model for ARHL. Supported by: NIH R01-DC-015988 (RR), NIH U24-DC020850 (AL), NIH T32-DC000023 (JM), and the David M. Rubenstein Research Fund.

T112. Slice and Dice: A Royal Road to Understanding Cochlear Micromechanics?

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Background: Optical coherence tomography (OCT) is now revealing fine-scale micromechanical motions of daunting detail and complexity. Perhaps the only way to make sense of the flood of new data—to determine, for example, which motions are truly important for cochlear function and which are merely ancillary—is to interpret them in the context of a model of comparable spatial resolution. Unfortunately, high-resolution cochlear models face a fundamental hurdle: With current technology their responses cannot be calculated in any reasonable amount of time. The field appears at an impasse.

But might there be a royal road to cochlear modeling that would allow one to resolve this impasse by speeding up the computations? For example, even though one cannot compute the response of a full, high-resolution model, one can compute the response of a short longitudinal section (or slice). Of course, computing the response of an isolated slice would only be useful if the resulting motions were similar to those observed in situ (i.e., when the slice is embedded within the full model). The question becomes: Is there a way to simulate the response of a full, high-resolution model by computing the response of a single, isolated slice? If the answer were an unequivocal "Yes", high-resolution cochlear models would immediately become useful in practice.

Methods: Recent studies undertaken with these considerations in mind have proposed the "Floquet method" as a means for using slices to solve high-resolution models. Here, we test the Floquet method by comparing model responses obtained from isolated slices with those of the corresponding full model. To facilitate interpretation, the cochlear models employed are kept simple (i.e., 2D active and passive linear models in which the organ of Corti is represented by a point impedance).

Results: We describe the Floquet method and discuss its limitations. When applied to cochlear slice models, the method yields mechanical frequency responses that differ dramatically from those of the full model, even when computed under near ideal conditions (e.g., full knowledge of the appropriate Floquet wavelength). Furthermore, small variations in model parameters yield changes in the slice frequency response that differ significantly from those produced within the full model.

Conclusions: We conclude by demonstrating a promising alternative method for accelerating the solution of high-resolution cochlear models. The method—which we optimistically dub the "Oquet method"—is based on theorems from the theory of differential equations about the existence and uniqueness of solutions and involves a systematic, iterated de- and refocusing of model detail. We outline the strengths and weaknesses of the Oquet method and discuss its possible application to understanding cochlear micromechanics in light of the emerging high-resolution data.

T113. Biasing Tone Level Steps Cause "Bounces" in Cochlear Status Revealed by Oscillatory Changes in DPOAE and SFOAE Modulation Side Lines

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Background: Previous research has shown that the exposure to loud low-frequency tones can cause oscillatory changes in cochlear sensitivity and otoacoustic emission changes immediately after the low-frequency exposure ceased. This oscillatory behaviour, which can last for a couple of minutes, was termed “bounce phenomenon”. Here we show that in gerbils a 60-Hz tone with relatively small level step from 75 to 90 dB SPL and back triggers similar “bounces”.

Methods: OAE and cochlear microphonics from the round window (RW CM) were recorded from deeply anaesthetised gerbils with synchronously gated primary tones and the 60-Hz biasing tone (BT). A sequence of 5-s presentations was presented in which alternating both primary tones ($f_1 < f_2$), or only the f_2 -primary tone were present. The latter evoked SFOAE, which was modulated by the 60-Hz tone). The inter-stimulus interval was < 0.5 s. During the first 50 alternating presentations, the BT-level was 75 dB SPL. During the next 50 presentations, the BT-level was increased to 90 dB SPL. The during the last 50 presentation, the BT level was again 75 dB SPL.

Results: Oscillatory level changes, typical for the “bounce” phenomenon were observed after both up- and down-steps in BT-level, mainly in the f_2 - f_1 DPOAE level, but also in the level of the closest modulation side line pair of the $2f_1$ - f_2 DPOAE and SFOAE. The oscillations were frequently as large as 10 dB. The polarity of the highly damped oscillatory changes was often primary level dependent. Despite the pronounced changes in the DPOAE spectrum, SFOAE changes were smaller and sometimes absent. The RW CM did not show any signs of a “bounce”.

Conclusions: Attempts will be shown to explain the changes in the OAE spectrum during the bounce by operating points shifts along the sigmoidal mechano-electrical transducer function of outer hair cells.

T114. Electrical Measurements in Turn Ii of the Gerbil Cochlea Before and After Disruption of the Endocochlear Potential

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Background: The endocochlear potential (EP), the approximately 80 mV potential difference between endolymphatic and perilymphatic spaces, is essential for cochlear amplification.

Previous experiments have used furosemide, which transiently abolishes EP, to study the loss and recovery of the active process in the cochlear base. Electromechanical responses return to baseline values by approximately two hours post treatment, while the EP recovers quickly, then stabilizes at a depressed value. We have extended these measurements to turn II of the gerbil cochlea where the best frequency (BF) is between 1.5 – 2 kHz.

Methods: Glass microelectrodes were inserted into scala media (SM) in turn II of gerbil cochleae and used to simultaneously measure both the steady-state and evoked potentials. The AC responses were corrected post hoc for the electrodes' low-pass filtering. Microphonic potentials (MPs) were measured in response to pure tones, 20 – 80 dB SPL, and the fundamental (first), second, and third harmonics were used to reconstruct smoothed time waveforms. These were fitted to a two-state Boltzmann function representing the mechano-electrical transduction (MET) input-output function and used to determine the operating point (OP). Tuning curves were also measured in response to multitone complexes from 0 – 80 dB. $2f_1 - f_2$ distortion products, both DPOAEs in the ear canal pressure and DPs in MPs, were measured in response to swept two-tone stimuli and were used to assess the cochlear condition and the efficacy of the drug delivery. Following baseline measurements, the EP was abolished with an IV injection of furosemide and an initial set of MP measurements (two-tone, complexes, and pure tones) was taken after the treatment. The measurements were repeated approximately every 15 minutes for up to four hours, to map changes in the OP.

Results: Baseline MP tuning curves were tuned to the local BF, with nonlinear responses at low-to-moderate SPL in the BF peak. At moderate-high SPL, nonlinearity extended sub-BF. After the IV administration of furosemide and the loss of the EP, the MP responses resembled those of a passive cochlea and largely recovered by about two hours post treatment. Relative changes in the ratio of the second harmonic amplitude to the fundamental over time were used to map changes in the OP. In preliminary experiments, the OP shifts from its baseline value and recovers on approximately the same timescale, about two hours, as the fundamental component of the MP, while the EP recovers more quickly, and stabilizes at approximately 40 mV.

Conclusions: Turn II is similarly affected by the loss of the EP as at more basal locations. Future experiments will use a double-barreled electrode to simultaneously measure the extracellular calcium, to further probe the role of MET in the recovery of cochlear operation.

T115. Outer Hair Cells Have Robust Motion Responses That Influence Reticular Lamina Behavior

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Background: Optical coherence tomography (OCT) has enabled in vivo sound-evoked motion measurements and imaging within the organ of Corti complex (OCC). While OCT has expanded our knowledge of how intra-OCC structures behave in response to sound, elements of the reticular lamina's (RL) contributions to the mechanics of the OCC are not fully understood. Here, we present three cases of RL motion behavior in gerbils with an interpretation of the cases using complex difference analysis and a simple model.

Methods: The OCT beam is directed through the round window membrane of the gerbil cochlea to obtain a transverse view of the OCC, where the best frequency (BF) is ~45 kHz. Basilar

membrane (BM), outer hair cell/Deiters' cell (OHC/DC) region, and RL motion are measured. The complex difference between the measured RL and BM motions extracts the internal motion of the RL that is "riding on" the motion of the BM. Using the internal RL motion, we employ a simple computational model to visualize the influence of the internal OHC motion on the measured RL motion.

Results: The OHC/DC region exhibits robust motion behavior. It is initially in-phase with the BM at low frequencies, then a gradual phase delay emerges as the frequency increases. Sub-BF, the OHC/DC region displays compressive nonlinearity, and the response magnitude is $>$ the BM response. The 80 dB sound pressure level OHC/DC response often exhibits a notch sub-BF and moves in phase with BM motion at frequencies above the notch. The RL response displayed three different types of behavior. In the first case, the RL phase has a small lead re: BM throughout the entire frequency range. The RL response magnitude is $>$ the BM response at and around the BF and $<$ the BM response sub-BF. In the second case, the RL phase initially lags the BM, then, still sub-BF, shifts to lead the BM. The RL response magnitude sub-BF is more similar to the BM and often exhibits expansive nonlinearity. In the third case, sub-BF, the RL phase is quite similar to the OHC/DC region, then becomes in-phase with the BM near the BF. In this case, there is often a notch in the RL response magnitude.

Conclusions: Complex difference analysis and a simple model are used to interpret these RL motion behaviors. The various behaviours can be understood as the RL-region being entrained to the robust OHC body response to various degrees. Compared to the BM, the RL is relatively flexible and can be influenced by the robust motion of active OHC bodies. An outstanding question is how this affects mechanoelectric transduction, which will depend on the RL and tectorial membrane and their stereocilia connection.

T116. Basilar Membrane Motion in Time Domain: Finite Element Study

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Background: A thorough understanding of the dynamic acoustic response of the auditory system is crucial. However, conventional research has largely been limited to frequency-domain analysis, focusing on steady-state responses to single-frequency inputs. This approach has inherent limitations in capturing complex dynamic behaviors, such as transient responses to initial sound stimuli or reactions to multi-frequency inputs. Therefore, this study aims to directly analyze the dynamic behavior of the cochlear basilar membrane (BM) in the time domain under complex acoustic stimuli.

Methods: A three-dimensional (3D) finite element (FE) head model, incorporating key anatomical structures including the skull, brain, skin, as well as the middle and inner ear, was utilized. To analyze the dynamic response in the time domain, sinusoidal sound pressures were applied to the tympanic membrane under two conditions: (1) a single frequency (100 Hz) and (2) multiple frequencies (0.5, 1, and 2.5 kHz). The pressure amplitude for each frequency component was set to 1 Pa. The time-dependent displacement of the BM was simulated from 0 to 1 second with a time step of 0.0001 s (0.1 ms) to analyze both transient and steady-state responses. For model validation, the amplitude and the location of maximum response from the

steady-state portion of the time-domain analysis were quantitatively compared with results from a frequency-domain analysis.

Results: The simulation predicted the time-dependent motion of the BM under both single and multi-frequency conditions. For the 100 Hz stimulus, the BM reached a stable steady state after an initial transient response of approximately 0.08 seconds, and this steady-state response was compared with frequency-domain results for model validation. Specifically, the maximum displacement amplitude for the 100 Hz stimulus showed an error of $< 5\%$ compared to the frequency-domain result. For the multi-frequency stimulus, the locations of maximum response for each frequency component also demonstrated an error within 4%. These findings validate the high accuracy and reliability of the proposed time-domain model.

Conclusions: This study is significant in its successful simulation and validation of the auditory system's time-domain response using a comprehensive head model. It provides an integrated framework for understanding complex auditory reactions, including responses to multi-frequency inputs and transient phenomena. The validated model can serve as a critical foundational tool for future research, such as predicting the biomechanical impact of sudden high-pressure acoustic events (e.g., blasts or impacts) on the auditory organs and elucidating the mechanisms of related pathologies.

T117. A 2DOF Cochlear Model Based on Critical Oscillators at the Hopf Bifurcation Predicts the Phase of Bm-RI Relative Motion

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Background: Critical oscillators were proposed for modeling the cochlear micromechanics due to some of their intriguing physical properties. Unfortunately, in a 1D limit, other properties seemed to poorly fit the actual cochlear phenomenology.

Recently, Ver Hultst (MOH 2024) demonstrated that critical oscillators can be successfully used for modeling the cochlear response if the 2D hydrodynamics is also included, providing the tall and broad activity pattern observed in the experiments. The combination between the critical oscillator behavior and the collective behavior due to the hydrodynamic coupling between the tonotopic micromechanical elements and the fluid may provide a coherent explanation for the dynamics of the Organ of Corti. In this study, the rather abstract formulation of the critical oscillator approach is interpreted in a realistic way, highlighting how it formally represents the response of an active low-passed system in a low-frequency regime. Indeed, two 2D hydrodynamic effects (focusing and viscosity) imply that the TW peaks in a region well basal to the “nominal resonant place”, where the elastic term still dominates the impedance.

Methods: A 2DOF transmission-line cochlear model was studied in the WKB approximation, with the RL and BM acting as two coupled oscillators, assumed equal for simplicity. The active force was modeled as an elastic internal force, low-pass filtered by the build-up of the transmembrane potential. One normal mode is associated to the center of mass motion and the other one to the relative motion of the oscillators. The active force only acts on the second mode, which, following Julicher, was modeled in time domain by two first order equations, describing the oscillator in the low-frequency limit, and the low-pass filtering of the internal elastic force.

By diagonalizing the system matrix, the system was put in the normal form of a critical oscillator at the Hopf bifurcation.

Results: The model was used to compute the motion of the BM and the RL, showing that the phase of the RL leads that of the BM before the best place region, approaching zero at the peak, and is sensitive to the activity level of the linear system. This behavior is in agreement with experimental data, and supports the hypothesis that fluid focusing plays a crucial role in anticipating the maximum peak of the traveling wave with respect to the tonotopic location. Without focusing, one would expect the BM and RL moving approximately in phase opposition in the peak region

Conclusions: Modeling the relative motion of the BM and RL in terms of a critical oscillators may offer a deeper insight into the physical properties of the system. Interpreting the properties of the micromechanical elements within that class of dynamical system may help understanding the meaning of the cochlear parameters tuning.

T118. Phase Reversal of Tectorial-Membrane Viscous Forces as a Mechanism of Cochlear Wave Amplification

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Background: Outer hair cells (OHCs) are central to the cochlear active process by injecting kinetic energy into the organ of Corti. However, how this energy couples to the nonlinear traveling wave is still an open question. We recently proposed that one pathway could involve the tectorial membrane (TM): OHCs would modulate TM deformations, thereby altering the viscous damping of the traveling wave. Here, we extend the proposed model by integrating the phase of the reticular lamina (RL) relative to the basilar membrane (BM). This extension reveals unexpected complex behaviors that could help explain cochlear amplification.

Methods: We assume that the viscous force acting on the organ of Corti can be expressed as the difference of viscous stresses on the lower and upper surfaces of the TM. With a few simplifying assumptions, the viscous force is found proportional to the second derivative of the transverse velocity across the TM thickness. Relative to the BM velocity, this results in a double dependence on the complex wavenumber k . Since k in turn is related to the BM admittance, this mutual relationship leads to a recursive definition of the wavenumber, which is resolved using an iterative procedure. Once k is determined, the nonlinear traveling wave can be simulated using the standard WKB approximation.

Results: Simulations reveal a critical value of the RL phase, denoted by θ_c , at which the system undergoes a sharp transition from maximum damping to negative damping. This shift in behavior is explained by TM deformations either leading or lagging the local transverse velocity, depending on the state of the system. Above θ_c , the viscous force arising from out-of-phase TM deformations can theoretically amplify BM motion. For a velocity field with positive curvature, as predicted by simple box models, the critical value is $\theta_1 = 0.25$ cycle. For a field with negative curvature, the critical value is lowered to $\theta_2 = -0.25$ cycle. The latter scenario may be physiologically realistic as an additional field with negative curvature could be induced by the three OHC rows via the TM collagen-fiber network. An intermediate configuration yields

continuous transition points with the critical phase gradually shifting from θ_1 to θ_2 in the peak region.

Conclusions: This work proposes an intriguing mechanism by which the active process controls the amount of traveling-wave viscous damping, and in some instances, even generates negative damping through friction. Within this framework, experimental RL-phase data appear consistent with the cochlea operating near a critical regime, where small phase shifts can cause large changes in damping. The model requires further validation, in particular by testing its predictions against experimental data from normal and TM-altered mutant rodents.

T119. Innovative Biomarker Discovery for Cochlear Hair Cell Injury: Optimizing Sample Collection and Preliminary Results

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Background: Sensorineural hearing loss (SNHL) often stems from cochlear hair cell injury, thus timely identification of readily measurable biomarkers associated with such injuries is vital for early diagnosis and intervention. Animal studies examining the pathophysiology of SNHL indicate that prior to notable increases in auditory brain stem response (ABR) thresholds, a loss of up to 30% of outer hair cells (OHCs) and up to 50% of inner hair cell synapses can occur. This presents a critical gap between standard audiological testing and detecting subclinical deficits on a molecular level before cochlear damage becomes irreversible. We hypothesize that biomarkers of early inner ear injury can be detected in both perilymph and plasma in mouse models for both genetic and acquired hearing loss by utilizing transcriptomic, untargeted metabolomic, and proteomic analyses. Conversely, biomarkers can also serve as markers of inner ear health – in particular for understanding gene therapy outcomes.

Methods: Three mouse models are used for this project. As a model of rapid OHC loss, we used RFX1/3;Gfi1Cre conditional knockout mice, in which OHCs begin to degenerate rapidly from postnatal day 12 (P12). Wild-type mice were used as controls. Cochlear tissue, perilymph, cerebrospinal fluid (CSF), and plasma were collected at P10 and P15, before and after the onset of OHC loss. Samples were also collected from CD1 mice for calibration analyses. As a model of acquired hearing loss, B6CBAF1/J mice were exposed to 97- or 105-dB SPL noise (8-16 kHz) for 2 hours to induce a temporary or permanent threshold shifts respectively. Samples were collected at 1 day post noise exposure. For Perilymph collection, the posterior semicircular canal (PSC) was exposed and sealed with surgical glue to minimize blood contamination. Adult mice

further required creation of a small window through the hardened glue by microsurgical knife to reduce contamination. Then, a 27- or 30-G needle was used to create a hole in the PSC through the hardened glue or the small window and perilymph was collected using a capillary needle. For CSF collection, the cisterna magna was exposed, and fluid extracted via capillary needle.

Results: The maximum volume of perilymph and CSF that could be collected from a single ear or animal was found to be 1 μ l or 10 μ l respectively. Preliminary metabolomic analysis for calibration showed that proline, nicotinamide mononucleotide, and L-carnitine were potential metabolites differentiating uncontaminated perilymph from CSF.

Conclusions: These optimized sample collection methods allow for the extraction of sufficient quantities of perilymph and CSF for metabolomic, transcriptomic and exosome analysis for inner ear health and injury. Ongoing and future experiments include, bulk RNA-sequencing using cochlear tissue samples and perilymph, CSF, and plasma analysis of metabolites and exosomes from all three mouse models listed in the methods.

T120. Investigating the Role of the Lipid Scramblase XKR8 in Inner Ear Function

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Background: Membrane lipid asymmetry is essential for cellular homeostasis, signaling, and programmed cell death. Caspase-activated lipid scramblases such as XKR8 mediate bidirectional phospholipid translocation across the bilayer, particularly during apoptosis, where externalized phosphatidylserine (PS) signals phagocytosis. While XKR8 is well characterized in immune and neural systems, its role in sensory tissues, including the inner ear, remains unclear. A dominant nonsense mutation in XKR8 (W237X, c.710G > A) has been linked to late-onset auditory neuropathy in humans and mice, but its mechanistic impact is unknown. Here, we investigate the localization, function, and genetic disruption of Xkr8 in the cochlea to better understand its role in auditory physiology.

Methods: We evaluated Xkr8 expression in the inner ear using RT-qPCR, RNA in situ hybridization, and immunofluorescence. Scramblase activity of wild-type and W237X-mutant XKR8 was assessed in Raji cells, which lack XKR8, via annexin V labeling and flow cytometry. We generated Xkr8^{-/-} mice by CRISPR-Cas9-mediated deletion of exon 3, and confirmed Xkr8 gene disruption by RT-qPCR. Auditory function of Xkr8^{+/+}, Xkr8^{+/-} and Xkr8^{-/-} littermates was assessed using auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs).

Results: Among the XKR-family of lipid scramblases (XKR4, XKR8, and XKR9), our RT-qPCR data show that XKR8 is the most abundantly expressed in the inner ear, with XKR4 present at significantly lower levels and XKR9 being absent. Lipid scrambling assays showed that XKR8 W237X is a loss-of-function variant that fails to traffic to the plasma membrane efficiently and externalize PS during apoptosis, even in the presence of its membrane chaperone Basigin. This defect likely results from the truncation of the XKR8 C-terminal transmembrane domains required for basigin interaction and membrane targeting. Despite the absence of Xkr8,

Xkr8^{-/-} mice exhibit normal ABRs and DPOAEs thresholds up to 30 weeks of age, suggesting no overt hearing loss. However, transcript analysis revealed only minimal upregulation of Xkr4 and Xkr9 in Xkr8^{-/-} mice, suggesting limited compensatory regulation. Thus, while an indirect mechanism cannot be ruled out as the cause of hearing loss in Xkr8^{W237X/+} mice, and given that Xkr4 is a more efficient scramblase than Xkr8, we generated Xkr4^{-/-}; Xkr8^{-/-} double-knockout mice to directly test functional redundancy. Auditory testing in these mice is currently ongoing.

Conclusions: This study advances our understanding of the roles of membrane regulatory proteins, particularly XKR8 and XKR4, in auditory function. Ongoing work aims to further elucidate the contribution of XKR8 to cochlear physiology and to define the broader role of XKR family genes in the genetic landscape of hearing loss.

T121. Characteristics of Dexamethasone Palmitate Lipid Nanoparticle in Protecting Against Kanamycin-Induced Ototoxicity: In Vitro and in Vivo Models

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Background: Drug delivery to the inner ear (IE) remains challenge. Dexamethasone (DEX) is widely used to protect against ototoxicity, but its conventional formulation, dexamethasone sodium phosphate (DSP), shows limited penetration after intratympanic (IT) injection. Nanoparticle-based formulations, such as dexamethasone palmitate lipid nanoparticle (DPLN), may enhance delivery and efficacy. DPLN, a liposomal formulation incorporating dexamethasone palmitate, has shown potent anti-inflammatory effects in chronic inflammation models and is clinically used for rheumatoid arthritis and osteoarthritis. This study compared the protective effects of DPLN with DSP in in vitro and in vivo ototoxicity models.

Methods: HEI-OC1 cells were cultured under permissive conditions and seeded in 96-well plates. After 3 h of serum starvation, cells were treated with kanamycin (5-20 mM) for 1-24 h to induce toxicity. Steroid pretreatment (1-200 uM, 6 h) was performed prior to kanamycin exposure. Cell viability and ROS levels were evaluated at multiple time points.

For in vivo experiments, adult C57BL/6 mice received subcutaneous kanamycin combined with intraperitoneal furosemide for two consecutive days to induce ototoxicity. IT-DPLN or DSP were administered every other day for a total of four injections. Auditory brainstem response (ABR) thresholds were measured at baseline and 7, 14, and 21 days after insult. Hearing gain was defined as the interaural threshold difference between the IT-injected and contralateral ears.

Results: In vitro, 24-h kanamycin exposure caused dose-dependent cell death, with 20 mM reducing viability by ~70%. With 20 mM kanamycin, steroid pretreatment improved viability, with the maximal protection at 10 uM DPLN (89.1%) and 100 uM DSP (90.7%). Notably, DPLN reduced early ROS spikes within 3 h post-kanamycin, reaching a maximum of 1.8-fold of control at 10 uM and 0.6-fold at 100 uM, suggesting rapid and sustained antioxidant action. In

contrast, DSP-treated cells showed elevated ROS, up to 2.6-fold at 10 uM and 3.0-fold at 100 uM in 3 h.

In the in vivo experiments, 400 mg/kg kanamycin with 170 mg/kg furosemide induced complete ototoxicity, producing a hearing loss of 82.5 dB SPL. IT-DPLN demonstrated greater auditory protection than IT-DSP, with hearing gains of 10 dB at 1 week and 16.7 dB at 3 weeks, compared with 6.7 dB at both time points for IT-DSP. Control ear showed minimal recovery.

Conclusions: DPLN exhibits stable antioxidant effects, mitigating ROS elevation and improving cells survival in vitro. Sustained release of active DEX likely underlies enhanced auditory protection in vivo, resulting in greater and longer-lasting hearing gains compared with DSP. The pronounced suppression of ROS by DPLN indicates its potential as alternative for IT-steroid therapy, effectively overcoming the limited penetration and viability efficacy of conventional DSP in the IE.

T122. Identification of Druggable Binding Sites and Small Molecules as Modulators of TMC1

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Background: Hearing and balance rely on the proper function of inner ear sensory hair cells, which convert mechanical stimuli into electrical signals via mechanoelectrical transduction (MET) channels formed by TMC1/2 proteins. Unfortunately, ototoxic drugs such as aminoglycoside antibiotics and cisplatin exploit these channels to enter hair cells, often causing irreversible auditory and vestibular dysfunction. Despite the urgent need for otoprotective therapies, our understanding of small-molecule interactions with TMC1 remains limited, impeding the development of targeted drugs. Structural insights into TMC1 and its binding partners provide a foundation for designing novel pharmacological modulators to mitigate drug-induced hearing loss.

Methods: The study implemented a structure-based drug discovery pipeline combining computational and experimental strategies. Using AlphaFold2 structural predictions of mouse TMC1 in complex with CIB2 and TMIE, we built an open-like conformation of the channel, embedded it in a POPC bilayer, and conducted molecular dynamics simulations to evaluate stability. Subsequently, ligand-based 3D pharmacophore models were developed from known MET channel blockers. These models guided pharmacophore-based virtual screening (PBVS) across millions of compounds from both commercial and FDA-approved drug libraries. Top hits were refined by docking and MM-GBSA free energy calculations, and a subset was experimentally tested using dye uptake assays in cochlear explants.

Results: The computational modeling identified three putative druggable regions along the TMC1 pore (top, middle, and bottom sites) where both known and novel compounds were predicted to interact. Several zwitterionic interaction networks involving residues D419, D528,

D569, and neighboring phospholipids were highlighted as critical for ligand binding. Pharmacophore screening yielded 207 non-FDA compounds and 53 FDA-approved drugs as promising candidates. Docking results confirmed consistent binding of classical blockers such as benzamil and tubocurarine, as well as novel hits including cepharanthine, posaconazole, and lapatinib. Experimental assays demonstrated that selected compounds effectively reduced dye uptake in mouse cochlear hair cells, confirming their ability to modulate TMC1 function.

Conclusions: This integrative approach combining pharmacophore modeling, molecular dynamics, docking, and in vitro validation establishes a versatile platform for discovering modulators of TMC1. The identification of multiple binding sites within the channel pore and validation of FDA-approved drugs as effective blockers highlights the translational potential of this work. By expanding the chemical space of candidate molecules, this study not only provides insights into the pharmacological modulation of TMC1 but also offers a rational framework for developing otoprotective therapies to counteract drug-induced hearing loss.

T123. Cellular Control of Functional Tonotopic Gradients in Cochlear Bone

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Background: Tonotopy of the basilar membrane arises from an EMILIN-2–dependent stiffness gradient. The surrounding cochlear bone—the stiffest bone in mammals—is essential for hearing, yet how its material properties are set is unclear. Because classic osteoblast/osteoclast remodeling is suppressed in the otic capsule, we hypothesized that osteocytes regulate cochlear bone quality along the cochlea’s established hearing axes. This builds on evidence that TGF- β /Runx2–dependent matrix calibration is required for normal hearing and that TGF- β inhibition can rescue hearing in Runx2 haploinsufficiency.

Methods: Cochlear bones from 4-month-old female C57BL/6J mice were profiled with a co-registered, multi-scale pipeline: quantitative backscattered electron imaging (calcium content), synchrotron μ CT (tissue mineral density and lacunar morphometry), second harmonic generation (collagen organization), polarization-dependent imaging contrast mapping (hydroxyapatite nanocrystal orientation), and nanoindentation (stiffness). Spatial proteomics by MALDI-QTOF MSI mapped extracellular-matrix peptides; immunofluorescence localized osteocyte/ECM markers. Bulk RNA-seq compared apical vs. basal halves. Notably, PIC-mapping and MSI were applied for the first time to bone to resolve nanoscale crystal orientation and spatial proteomic gradients.

Results: Cochlear bone properties varied coherently across radial and tonotopic axes. Radially, inner cortex was more mineralized than outer cortex at apex, middle, and base. Tonotopically,

the basal turn showed higher mean mineral density than the middle. At the nanoscale, adjacent nanocrystal misorientation increased from apex to base, while collagen alignment decreased basally; stiffness tracked mineral content most strongly in lamellar regions. Lacunae were larger and more elongated apically and smaller/rounder basally and inwardly, consistent with heightened apical perilacunar/canalicular remodeling (PLR). Immunofluorescence confirmed inner-layer enrichment of DMP1, osteopontin, and MMP13. Spatial proteomics identified basal enrichment of TGF- β antagonists (Asporin, EMILIN-1). RNA-seq revealed apical TGF- β pathway enrichment with basal up-regulation of antagonists (e.g., *Aspn*, *Dcn*, *Ltbp4*, *Fmod*, *Cilp*). Together, these datasets define a spatially patterned TGF- β program that directs osteocyte PLR and ECM composition, establishing axis-registered gradients in cochlear bone material properties that mirror functional hearing axes.

Conclusions: Cochlear bone behaves as a patterned, functionally graded material that likely co-tunes with sensorineural tissues to support frequency selectivity and gain. An osteocyte- and TGF- β -based program appears to sculpt these gradients, and a shared toolkit—EMILIN-1 in bone mirroring EMILIN-2 in the basilar membrane—may coordinate tuning across hard and soft tissues, supporting integrated cochlear function. In light of prior evidence that TGF- β /Runx2-dependent matrix calibration is necessary for hearing and pharmacologically adjustable, our findings suggest a working causal model linking bone material quality to auditory function and point to osteocyte PLR and local TGF- β tone as testable, potentially modifiable levers in sensorineural/mixed losses (e.g., cleidocranial dysplasia, otosclerosis).

T124. Gper1 Localization in the Murine Organ of Corti

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Background: There are well-documented sex differences in hearing in human populations, including differences in cochlear nerve function. For example, auditory brainstem response (ABR) recordings show a greater wave 1 amplitude and shorter latency in females compared to age-matched males. Wave 1 amplitude represents the total electrical activity of the cochlear nerve, while the latency represents the speed of neurotransmission. These components of the ABR are generated by the summed activity of the glutamatergic synapse between the inner hair cell and spiral ganglion neurons, of which the AMPA receptor dominates at the post-synaptic density. In mice, there is evidence that females express a higher ratio of fast gating GluR2 to slower GluR3 AMPA receptor subunits than age-matched males, likely underpinning, at least in part, the faster wave 1 latency exhibited in females. Interestingly, the wave 1 amplitude and latency appear stronger in females during points in the ovarian cycle when estrogen peaks, suggesting that estrogen signaling may mediate neurotransmission in the auditory periphery. Studies have shown that the g-protein coupled estrogen receptor (GPER1) has a role in mediating AMPA receptor insertion into the post-synaptic density in the hippocampus, thereby enhancing long-term potentiation. Based on these data, we hypothesize that GPER1 is responsible for facilitating an estrogen-mediated effect on synaptic transmission in the inner ear.

Methods: We used immunohistochemistry to localize Gper1 in the adult mouse cochlea. Spiral ganglion neurons (SGN) were visualized with an neurofilament antibody, anti-Myosin VIIa was

used for visualizing the inner hair cells (IHC), and anti-GPER1 to locate the Gper1 puncta. Labeled cochlea were imaged on a confocal microscope with 63x magnification.

Results: Gper1 puncta appear clustered at the IHC synaptic junction and distributed along the SGN axon. Puncta are also present in the OHC compartment, where they are much less common and do not appear in every cell.

Conclusions: Our results clearly show that Gper1 is expressed in the mammalian organ of Corti, consistent with prior studies showing Gper1 RNA expression in the post-natal murine cochlea. Furthermore, Gper1 localization to the SGN provides evidence for participation in synaptic transmission and suggests a mechanism by which estrogen may mediate sex differences in the inner ear. Future work will further characterize the role of GPER1, sex, and phase of estrus cycle as modulators of cochlear neurotransmission.

T125. Evaluation of the Hearing of Junctophilin3 Knockout Mice

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Background: Junctophilin3 (JPH3), a calcium homeostasis modulator has been implicated in Huntington's Disease Like-2, in which JPH3 expression is lower than normal subjects. Junctophilin3, a protein forms a stabilizing junctional membrane complex between the plasma membrane and endoplasmic reticulum. It plays an important role in a Cav1-RyR2-KCa3 complex by forming intracellular calcium microdomains to modulate Ca homeostasis. JPH3 has a wide distribution in the brain including high expression in cortex, striatum, cerebellar and inferior colliculus. Despite low expression in HDL2, hearing loss has not been reported, however, impaired auditory startle and prepulse inhibition (PPI) were detected in Huntington's disease and JPH3 knockout mice.

Methods: Auditory brainstem-evoked responses (ABRs, BioSigRZ, TDT) were recorded in both C57/BL6 and JPH3 knockout mice using click and tone-burst stimulus, following DPOAEs tests. The stimulus of 20 to 90 dB stepped by 5 dB were used to induce both ABRs and DPOAEs. Local field potential (LFP) oscillations were further evaluated (RZ6, TDT) by in vivo neurophysiology in the auditory cortex and striatum.

Results: ABRs were evaluated in two age groups. In 4-6 weeks, the thresholds of C57 mice were 25 ± 1.5 dB ($n = 11$), the hemizygous JPH3 Knockout were 32.9 ± 4.7 dB ($n = 7$), the homogenous JPH3 were 42.9 ± 4.6 dB ($n = 19$). In 12-14 weeks, the thresholds of C57 were 26.4 ± 1.2 dB ($n = 11$), JPH3 Het were 34 ± 4.1 dB, JPH3 Hom were 39.8 ± 4.8 dB ($n = 14$). This demonstrates that the JPH3 Hom mice showed significant hearing loss ($p < 0.05$). The thresholds of DPOAEs for the C57 mice at 24 Hz were 38.8 ± 3.4 dB and 57.5 ± 7.5 dB ($n = 5$) for 32 kHz in 12-14 groups, but for JPH3 Hom Mice, 24 Hz: 73.3 ± 11.7 dB; 32 Hz: 86.7 ± 1.7 dB ($n = 3$). Under the anesthesia of urethane, the oscillation pattern in striatum across all the spectral bands were tested (Theta, 6 - 12 Hz; Beta, 15 - 35 Hz; low gamma, 40 - 80 Hz; high gamma, 100 - 140 Hz; HFO, 160 - 190 Hz). Specifically, both low and high gamma oscillations are affected by JPH3 knockout. In consistent the findings in dorsal striatum, JPH3 knockout also reduced gamma oscillation activity in the auditory cortex.

Conclusions: We have found that there is hearing loss in JPH3 KO Hom but not Het mice, which is associated with modified LFP oscillations. This implicates that the hearing loss is caused through both central and peripheral mechanisms. It suggests that JPH3 is a risk factor of hearing loss, which might be the underlying mechanism for impaired PPI in Huntington's disease and JPH3 KO mice.

T126. Fine Ultrastructure of Somatosensory Inputs in the Granule Cell Domain of Dorsal Cochlear Nucleus

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Background: The granule cell domain of the cochlear nucleus (GCD) receives mossy fiber inputs from multiple brain regions. This circumstance situates the GCD for integrating vision, balance, and proprioception at the earliest stages of auditory processing. The polysensory inputs help segregate sound streams in three dimensional space. The structural substrate for this function is elaborate. Mossy fibers of different origins are large, multilobed endings that form synaptic glomeruli with postsynaptic targets that include granule cells, unipolar brush cells, cartwheel cells, and chestnut cells, which in turn project to principal cells in the dorsal cochlear nucleus. Numerous synapses contribute to the integration of the different modalities, but the organization of these synapses is not well understood.

Methods: We addressed this issue by labelling the presynaptic endings of different somatosensory inputs using biotinylated dextran amine or Phaseolus vulgaris leucoagglutinin and studying their postsynaptic targets in the GCD. The anterogradely-labeled endings and associated targets were visualized by light and electron microscopy.

Results: Following three-dimensional reconstruction, we found that unipolar brush cells are the main targets of the C2 DRG and cuneate nucleus. Mossy fibers from the spinal trigeminal nucleus project to Golgi cells, an inhibitory neuron, whereas inhibitory boutons originated from the lateral reticular nucleus.

Conclusions: These results reveal that somatosensory integration in the granule cell domain is achieved by distinctive synaptic wiring and specialized chemical signaling.

T127. Auditory-Motor Surprisal Reveals Context-Sensitive Dynamics in a Novel Music Task

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Background: Auditory-motor learning is important in learning how to produce and control sounds such as in speech and playing music. As in all sensorimotor learning, it is based on internal models of interactions between actions and their sensory consequences, which are fine-

tuned by minimizing the errors ("surprisal") between the received sound and those predicted from the internal model.

Methods: Here we apply the concept of surprisal to probe the neural dynamics of sensorimotor learning in the context of a keyboard-playing task in which participants played on a keyboard that changes between three key-to-pitch maps in random order and at short time intervals while their EEG was recorded during playing, listening, and miming (playing the keyboard without sound). We hypothesized that staying within the same key-to-pitch map engenders the smallest errors (surprisals), compared to switching between different maps.

Results: We extracted a neural signature of auditory-motor surprisals by comparing first keystroke after a map change (highly surprising events) to all other keystrokes (less surprising events). We found a signature of auditory-motor surprisal, the N100, which is not fully explained by pure auditory surprisal. Further analyses showed that this surprisal signature is found in the auditory response rather than the motor response. The magnitude of N100 is modulated by short-term context, with greater surprise after a long period without surprising events, showing that in addition to using short-term context to predict the auditory-motor mapping, the brain tracks environmental volatility in real time. Long-term musical training further enhances N100 amplitude. In contrast, P50 amplitude is modulated by 30-minutes of goal-directed training, and is apparent only in the auditory-motor mapping that was trained.

Conclusions: This experiment presents a novel approach to studying auditory-motor surprisal using time-varying stimuli from the perspective of online surprisal. Our findings reveal that auditory-motor learning is fast, context-sensitive, and shaped by both short- and long-term experience.

T128. Investigating the Impact of Audiovisual Manipulation on Movement and Balance in Realistic VR Tasks: A Feasibility Study

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Background: There is growing interest in conducting ecologically valid studies to assess the interactions among the hearing, vision, vestibular and movement systems because they are linked. Previous research in this area has been limited to artificial tasks and imprecise measurements of movement and balance. Virtual reality (VR), combined with motion capture (MoCap), offers a safe and reliable method for studying the impact of hearing loss on ecologically valid, day-to-day activities.

The aims of this study were to determine whether VR and MoCap can be used to study the influence of hearing on movement and balance.

Methods: Following input from two Patient and Public Involvement (PPI) sessions, four VR tasks were developed and tested:

* A 'bus ride' task investigated anticipatory postural changes in response to auditory cues to movement. The task simulated a bus ride during which participants were standing passengers holding on to a static pole for support. Variations in the intensity of the engine, horn, and brake

sounds signalled acceleration, deceleration, and a change in direction of the bus. Participants' body sway, and force on the pole were assessed.

* A 'corridor' task investigated the impact of auditory distraction on ambulatory performance. Participants navigated a winding corridor with various obstacles while engaging in active or passive listening tasks. Participants' body sway, number of steps, physiological arousal and obstacle collisions were assessed.

* A 'kerb walk' task investigated threat avoidance in response to startling auditory cues. Participants walked along the edge of a kerb while an ambulance passed by from behind, with sirens presented gradually (indicating vehicle approaching) or suddenly (reduced cues to approach, inducing a startle response). Catch trials were included to prevent anticipation. Participants' body sway, number of steps, and physiological arousal were assessed.

* A 'road crossing' task investigated the impact of manipulating auditory and visual cues on ambulatory performance, hesitancy, and successful task completion. Participants attempted to cross a busy road while the speed of approaching vehicles, weather conditions, and background noise were manipulated to alter task difficulty. Participants' body sway, number of steps taken, number of head turns, false starts, and car collisions were assessed.

Results: Data collection using the VR tasks and MoCap is ongoing at the Virtual Reality Research (VR2) facility, The University of Manchester, UK.

Conclusions: Our ongoing pilot study will provide evidence of the feasibility of using VR and MoCap as acceptable and valid methods for assessing the impact of hearing loss on balance and movement.

T129. Altered Temporal Processing in the Inferior Colliculus of Mice Lacking the Extracellular Matrix Protein Brevican

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Background: The proteoglycan brevican is a major component of the extracellular matrix of perineuronal nets and is highly enriched in the perisynaptic space suggesting a modulatory role in synaptic transmission. Brevican is part of perineuronal nets at various stations of the auditory pathway, e.g. at inner hair cells synapses and at the somata of bushy cells, octopus cells in the cochlear nuclear complex, medial nucleus of the trapezoid body (MNTB) neurons, medial superior olive (MSO) and lateral superior olive (LSO) neurons, neurons of the dorsal nucleus of the lateral lemniscus (DNLL) and some neurons of the inferior colliculus (IC) and the auditory cortex (AC). We have studied the role of brevican for spectral and temporal coding in the IC by using brevican knockout (bcn^{-/-}) mice.

Methods: To this aim, we performed in vivo electrophysiological recordings from neurons of the IC from systemic bcn^{-/-} and bcn^{+/+} littermates. Responses of IC neurons to pure tones and amplitude-modulated (AM) tones were recorded and characterized as a function of modulation frequency.

Results: Hearing thresholds, spontaneous rate, and frequency tuning characteristics of IC neurons of bcn^{-/-} mice were normal. Lack of brevican, however, led to shorter first spike

latencies of neuronal responses at modulation frequencies ≥ 70 Hz and to two- to threefold evoked rates in response to AM tone stimulation. Surprisingly, the vector strength and the corresponding correlation coefficients of phase locking ≥ 50 Hz modulation frequency were increased in *bcan*^{-/-} mice compared to *bcan*^{+/+} littermates.

Taken together, our results demonstrate that lack of the extracellular matrix protein brevican improves rather than impairs auditory processing of AM tones.

Conclusions: So far, we cannot explain these puzzling results. One problem of this study is the systemic deletion of brevican. Experiments using conditional, region-specific knockout mouse models would help to understand the effects of brevican in the auditory pathway in more detail.

T130. Predicting Auditory Midbrain Responses to Natural Sounds With Integrate-And-Fire Gabor Receptive Field Models

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Background: Spectro-temporal receptive fields (STRFs) are widely used to model the time-frequency sensitivity of auditory neurons. Often, STRFs are derived using unbiased synthetic stimuli, such as dynamic ripples or random chords, which can easily be estimated using spike-triggered averaging. However, with natural sounds, decorrelation and regularization techniques are needed to remove embedded stimulus correlations that distort the estimated STRFs.

Furthermore, nonlinearities and non-stationarities make it difficult to predict spiking neural responses to natural sounds at millisecond timescales.

Methods: Neural recordings were obtained from the inferior colliculus (IC) of unanesthetized rabbits in response to an ensemble of 50 natural sounds as well as dynamic moving ripples (DMR). We developed a model-based approach for deriving auditory STRFs and predicting single trial spike trains to these sounds. The model consists of a first and second-order nine parameter Gabor STRF (gSTRF; Qiu et al. 2003), accounting for the neuron's linear and non-linear spectro-temporal integration. A four-parameter nonlinear integrate-and-fire (IF) compartment incorporates intrinsic noise, cell membrane integration, and nonlinear thresholding to generate simulated spikes, and a six-parameter adaptive context receptive field that dynamically adjusts the intracellular current to account for slow fluctuations in firing rate in response to naturalistic stimuli. Simulated annealing optimization was used to fit neural data and derive optimal model parameters by minimizing the log-likelihood.

Results: We compared optimal gSTRFs to other approaches such as a generalized linear model and regression. STRFs derived via regression were spectrally smeared, indicating that stimulus correlations were not effectively removed. In comparison, the gSTRF was compact and provided biologically feasible parameter estimates, including best frequency and temporal modulation. The adaptive context receptive field improves predictions of firing changes across sounds not captured by the gSTRF alone. Furthermore, unlike conventional receptive field models, which generate continuous outputs, the model produces and predicts spiking activity with spike timing precision comparable to IC neurons (between ~ 0.5 - 5 ms).

Conclusions: This new approach allows one to derive auditory STRFs and predict neural spiking activity to natural sounds in IC using functionally interpretable basis functions. The small

number of functionally interpretable parameters makes exploration of nonlinear and nonstationary effects due to natural sound statistics more feasible.

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T131. The Influence of Spectrum and Modulation Statistics on the Neural Representation of Speech in Natural Background Sounds

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Background: Separating target sounds from background noise is challenging for people with normal hearing, and especially challenging for people with hearing impairments as well as artificial speech recognition systems. Why different natural backgrounds mask the perception of speech to varying degrees, and how the brain separates speech from background noise is not yet fully understood.

Methods: Here, we recorded multi-unit activity from the inferior colliculus of head-fixed unanesthetized rabbits passively listening to speech mixed with eleven natural backgrounds (speech babble, bird babble, water, fire, city noise, etc.). Speech was mixed with each original (OR) background at multiple acoustic signal-to-noise ratios (aSNRs). We also presented speech mixed with spectrum-matched (SM) backgrounds that retain the original spectra but have whitened modulations, or modulation-matched (MM) variants that retain the original modulations but have whitened spectra. Using a cross-spectrum shuffling procedure, we separated the foreground- from the background-driven neural responses. The neural signal-to-noise ratio (nSNR) was then estimated and used as a metric of speech coding fidelity.

Results: We find that nSNR depends on background category and statistics. Compared to OR backgrounds, nSNR can increase or decrease for SM and MM backgrounds in a category-dependent manner. This suggests that the original spectra (OR vs. MM) or modulations (OR vs. SM) improve or worsen the neural representation of speech. Interestingly, the impact of background category and statistics was largely independent of aSNR, since increasing aSNR amplified nSNR approximately linearly. We also demonstrate how the background spectrum and modulation statistics affect neural encoding of speech at temporal modulations below 10 Hz, which overlap and potentially mask temporal fluctuations for phonemes, words, and syllables. Spectrum and modulation masking effects were segregated by background category, and a model of the cochlea and auditory midbrain showed similar segregation. Finally, human perceptual tasks using the same backgrounds suggest that nSNR correlates with speech recognition accuracy.

Conclusions: Collectively, the results posit that spectrum and modulation statistics critical to speech masking are reflected in the population activity of the inferior colliculus. These neural response statistics reflect the coding fidelity for speech and likely contribute to downstream signaling underlying speech perception in real-world noise.

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T132. Encoding of Masked Amplitude Modulation in the Inferior Colliculus of the Budgerigar

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Background: Amplitude modulation (AM) is an important acoustic feature of speech, animal vocalizations, and many real-world listening environments. Human studies support a modulation filterbank model for AM processing in noise, which separate target AM signals from noise based on differences in AM frequency. However, the neural bases of the modulation filterbank model remain unclear. The inferior colliculus (IC) in the midbrain is a key brain hub for processing AM. However, previous studies have not examined IC encoding of AM in the presence of AM maskers. The present study recorded single-unit IC responses to masked AM in budgerigars to test the neural bases of the modulation filterbank hypothesis.

Methods: Single-unit neural responses were recorded in the central IC of anesthetized budgerigars. Modulation transfer functions (MTFs) were measured in responses to AM tones with 100% modulation depth and the carrier frequency equal to characteristic frequency (CF) of the neuron. Modulation frequency was then varied to determine excitatory and inhibitory AM frequency ranges with respect to an unmodulated CF tone. Masked AM responses were recorded for stimuli containing both a target AM signal and AM masker. Carrier frequency was equal to CF, and the target AM frequency was selected to occur within an excitatory or inhibitory region of the MTF. Target AM depth ranged from -20 (10%) to -6 dB (50%). Masker AM frequencies were within 2 octaves of the target AM and had -6 dB modulation depth. Signal detection theory was used to estimate the minimum modulation depth for neural detection of the target AM across AM masker frequencies, for direct comparison to behavioral AM masking patterns.

Results: MTFs of the budgerigar IC neurons commonly showed AM frequency ranges with excitation, inhibition, or both excitation and inhibition in responses to AM tones. Many IC neurons had MTFs with band-pass characteristic tuning. MTFs of some neurons showed substantial excitation or inhibition over a relatively narrow AM frequency range, while MTFs of other neurons were less sensitive to specific AM frequencies. Masked AM responses showed diverse patterns partially explainable by the MTF. Target AM frequencies in excitatory region were detected based on rate increase whereas AM frequencies in inhibitory region were detected based on a rate decrease. In general, results suggested increasing target AM detection thresholds (i.e., decreasing AM sensitivity) for masker AM frequencies approaching the target AM frequency.

Conclusions: Preliminary results show IC neural detection patterns for masked AM that are broadly consistent with the modulation filterbank hypothesis, including decreasing target AM neural sensitivity for masker AM frequencies closer to the target AM frequency. These results provide new insight into the neural bases for perception of AM signals in real-world environments with fluctuating noise.

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T133. Serial Monitoring of Hearing With Otoacoustic Emissions: Effect of Stimulus Calibration

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Background: Recent work from our laboratory demonstrates that forward pressure level (FPL) stimulus calibration for otoacoustic emissions (OAEs) improves short-term test-retest repeatability of distortion-product OAEs (DPOAEs) at high frequencies, where ear-canal standing waves are most troublesome. This may enhance the ability to detect meaningful changes in OAEs when monitoring hearing and hearing loss. Here we: (1) Define the long-term test-retest reliability of the nonlinear component of the DPOAE and the stimulus-frequency OAE (SFOAE), both evoked by swept tones calibrated in dB FPL; (2) Compare the reliability of swept-tone DPOAE to that of conventional discrete-tone DPOAE (calibrated in dB SPL) in the same ear; and (3) probe the performance of a joint distortion + reflection OAE profile in detecting hearing shifts.

Methods: We tested 20 subjects, 16 of whom had a history of moderate (music-related) noise exposure. Fifteen had normal-hearing (≤ 20 dB HL) and 5 had slight-mild hearing loss. We measured swept-tone DPOAEs and SFOAEs with stimuli calibrated in dB FPL and conventional discrete-tone DPOAEs with stimuli calibrated in dB SPL every two months for one year. Reliability metrics were calculated for each OAE: absolute level differences upon repeated testing, the standard error of the measurement (SEM), and the 95% reference shift-limits, which define normal level variation over time. Additionally, because 7 thresholds worsened over the test year (≥ 15 dB), we compared OAEs for these shifted thresholds with the 95% reference shift-limits to assess which emission could best detect changes in hearing.

Results: Absolute level differences and SEMs between repeated test sessions for the conventional discrete-tone DPOAE (SPL) were significantly larger than those of the swept-tone DPOAE (FPL). For normal-hearing individuals returning for repeated tests, we expect swept-tone DPOAEs (calibrated in dB FPL) to be within ± 3 -4 dB of their original levels (from 1-6 kHz) and conventional discrete-tone DPOAEs (calibrated in dB SPL) to be within ± 6 -8 dB. When the derived 95% reference shift-limits were applied to the 7 instances of worsened hearing, swept-tone DPOAEs combined with SFOAEs identified hearing shifts in 4 of the 7 cases; the conventional DPOAE identified none.

Conclusions: The nonlinear component of the swept-tone DPOAE evoked with tones calibrated in FPL was more repeatable than the discrete-tone DPOAE (SPL) measured in the same ear. The greater reliability suggests that swept-tone DPOAEs calibrated in dB FPL can detect the progression of hearing loss earlier than conventional DPOAEs. A preliminary, small-scale assessment was consistent with this hypothesis when DPOAE and SFOAE results were combined. Monitoring and tracking hearing and hearing loss with OAEs appears to benefit from FPL calibration and from measuring reflection and distortion emissions together.

T134. Hearing Your Eyes Move: A Tensor Tympani Myoclonus Case Study

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Background: Eye movements are critical to linking vision and spatial hearing. Sounds are localized based on cues anchored to the head/ears while the locations of visual stimuli are detected relative to the orientation of the eye. When the eyes move relative to the head, the relationship between the native visual and auditory reference frames shifts. We do not normally perceive this, indicating that the brain must be incorporating information about eye movements into the process of localizing and integrating auditory and visual stimuli.

Indeed, we and others have found evidence that the brain sends signals about eye movements to the ears, producing a novel type of otoacoustic emission called an eye movement-related eardrum oscillation (EMREO) (e.g., Gruters et al, PNAS 2018; Brohl and Kayser, J Neurosci 2023; Abbasi et al, Hearing Research 2025). In most participants, EMREOs would be expected to be too quiet to be heard – the median peak-amplitude sound level is about 55 dB SPL (Lovich et al PNAS 2023), which is below threshold for humans in this low frequency range (30-50 Hz). However, people routinely contact us about this work because they do hear a sound when they move their eyes. Here we report findings from one such participant, following up on our initial analysis of this subject (King et al ARO 2024).

The three overarching questions of interest are 1) what exactly is the patient hearing, 2) how are their EMREOs different from those of other participants, and 3) what is the underlying cause of these differences? Taking the last question first, the relevant clinical history of this patient involves suspected tensor tympani myoclonus, i.e., involuntary spasms of the tensor tympani muscle – thus any atypical features of EMREOs in this patient help shed light on the role played by this muscle in EMREOs. Indeed, recent animal and human clinical work suggests that the tensor tympani normally dampens EMREOs (Lovich et al, SimCo 2025; King et al, MoH 2024). Results from this patient are consistent with this pattern.

As for the first two questions, our previous evaluation of this patient was somewhat puzzling because the observed EMREOs, while large than normal, were not fully outside the range of those observed in participants who do not report hearing sounds associated with eye movements.

Methods: Accordingly, we expanded the analysis in both the frequency and time domains

Results: We report here two new findings: a prolonging of eye movement related eardrum signals relative to the saccade, and increased energy not only in the typical EMREO band (30-50 Hz), but also at a lower frequency band, specifically 10-30Hz.

Conclusions: We suspect this increased EMREO duration and spectral bandwidth contributes to why this participant hears sounds when they make eye movements.

T135. The Inner Ear's Active Process Contributes to Selective Attention to Speech in Noise

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Background: Humans excel at understanding speech in noisy environments by selectively focusing on a target sound while suppressing competing signals. Although auditory stream segregation is often attributed to the auditory cortex, descending pathways connect higher auditory centers to the brainstem and cochlea, allowing modulation of peripheral responses. Otoacoustic emissions (OAEs) - faint sounds generated by the basilar membrane's nonlinear

mechanics - provide a noninvasive measure of cochlear activity. Distortion product OAEs (DPOAEs), typically evoked by two pure tones, have rarely been studied with speech-like stimuli. Here, we used speech-derived DPOAEs (speech-DPOAEs) to test whether selective attention modulates peripheral responses, and whether effects vary between resolved and unresolved harmonics, which differ in their spatial separation along the basilar membrane.

Methods: Stimuli were synthesized from male and female speech, following harmonic overtones n and m ($n < m$) of the fundamental frequency to elicit cubic distortion products at the overtone $2n-m$. Resolved harmonics are expected to produce distinct peaks along the basilar membrane; unresolved harmonics likely overlap substantially. Stimuli were designed for simultaneous measurement of speech-DPOAEs to both voices. Forty normal-hearing adults (18–31 years) listened to two competing voices in one ear while stimuli and DPOAEs were presented and recorded in the contralateral ear. Participants alternated attention between the male voice, the female voice, or a visual distractor. Each attentional condition was repeated across multiple trials.

Results: Speech-DPOAEs from resolved harmonics were significantly reduced when the corresponding voice was attended compared to when it was ignored. No such effect was observed for unresolved harmonics when both target and competing voices contained unresolved components in the same frequency range. This pattern suggests that attention selectively modulates spectrally resolved speech elements. Similar modulation emerged when auditory attention was compared to visual attention, indicating that intermodal attention can also influence cochlear responses. Further details can be found in the preprint of the publication, available at DOI: <https://doi.org/10.1101/2025.08.15.670505>

Conclusions: These findings provide the first direct evidence that selective attention to speech in noise can modulate the cochlea's active process, influencing peripheral auditory encoding. The observed specificity to resolved harmonics aligns with known cochlear tuning properties and supports the idea that attentional control operates at multiple levels of the auditory hierarchy, extending to the periphery. The speech-DPOAE paradigm introduced here enables simultaneous, frequency-specific measurement of cochlear responses to multiple voices, offering a novel tool for studying auditory scene analysis under ecologically valid conditions. Future work will systematically investigate how harmonic structure and fundamental frequency shape attentional modulation, with potential implications for clinical assessment of auditory processing in complex listening environments.

T136. Oto coherence: Interspecies Analysis of Phase Self-Consistency in Spontaneous Otoacoustic Emission

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Background: Spontaneous otoacoustic emission (SOAE) provides compelling evidence of active force generation inside the inner ear, although significant debate exists about the underlying generation mechanisms. SOAE is commonly characterized by peaks in the spectral magnitudes, which occur at frequencies unique to a given ear. This measure is typically computed as an averaged magnitude spectrum that discards phase information. Here, we explore

the hypothesis that this phase information reveals pertinent features of SOAE generation beyond what can be extracted from magnitudes alone.

Methods: Our method characterizes the self-coherence of oscillations over time. This “otocoherence” uses a short-time Fourier transform to provide a set of phase estimates as a function of both frequency and time. For each frequency, we calculate the phase accumulation over time periods of some fixed length. The consistency of these phase accumulations is then quantified, providing an otocoherence spectrum: a function of frequency analogous to the magnitude spectrum, but calculated using only phase information from a single SOAE signal. For a fixed frequency, otocoherence is a function of the length of the accumulation time period, typically falling off roughly exponentially as this time increases. Fitting an exponential to this decay allows us to extract a time constant, a process we repeat for a handful of SOAE frequency centers per subject. These data allow us to characterize the self-coherence of spontaneous emission as a function of frequency across species. Finally, we introduce a class of dynamic windowing methods to address issues associated with the fundamental time-frequency tradeoff.

Results: We analyzed SOAEs recorded from humans, barn owls, and lizards, and found striking interspecies differences in otocoherence. Our comparison of the otocoherence decay constants with measures of tuning extracted from auditory nerve fibers (barn owl and Tokay gecko) indicated that the method can predict the upper bound of frequency selectivity encoded in the central nervous system. Since our approach does not involve presenting any external stimuli, our results indicate that measures of the spontaneous emissions on their own allow for a noninvasive and stimulus-independent way to infer the degree of cochlear tuning possible.

Conclusions: We report that SOAE phase information provides data that extend beyond spectrally averaged magnitudes alone. A key finding is that the decay in otocoherence at a fixed SOAE frequency appears to allow for estimates in peripheral auditory tuning. These methods can readily be employed to re-analyze previously collected SOAE waveforms from a variety of species, making them of broader comparative utility. Further, we suggest how these methods can be extended to reveal new information about SOAE generation. For example, otocoherence-based methods can provide a complementary tool to improve the detection of small-magnitude SOAE peaks near the noise floor.

T137. The Neural Basis of an Innate Preference for Conspecific Vocalizations

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Background: During development, the brain uses sensory input to shape the organization of neural circuits underlying learned behavior. There is considerable evidence that inherited traits can prime the brain to encode behaviorally-relevant information during development. How genetic background influences the early learning process and shapes future behavior is unknown. Here, we studied songbirds to identify mechanisms by which sensory biases in auditory processing emerge from genetic background and experience. Juvenile songbirds learn to sing by listening and copying their father’s song, followed by practice to develop their own songs, which become stereotyped and stable by adulthood. Considerable work across species has found that the juveniles of both sexes prefer to hear the conspecific song over heterospecific song, even before they are exposed to singing adults. This auditory preference suggests that the juvenile

songbird brain is genetically-primed to attend to the acoustics features of conspecific song, prior to any experience. However, if raised by heterospecific tutors, song preference can shift from conspecific song, indicating an interplay between genetic background and early-life experience in song development. Here, we assess and compare neuronal tuning for song acoustics in the sensory-motor stage of song learning, in two songbird species, and whether it is reversed by early-life experience.

Methods: I performed electrophysiological recordings of the auditory forebrain of zebra finch (*Taeniopygia guttata castanotis*) and Bengalese finch (*Lonchura striata* var. *domestica*), two closely related species with dissimilar song acoustics. Recordings were performed in juvenile males raised in an acoustically enriched environment. Recordings were performed in awake juveniles between 60-80 dph, during the time that the songbirds began developing their own song. Stimuli consisted of tones, spectrotemporal modulations, and the song of the juvenile's father and an unfamiliar conspecific. Tones were used to verify normal hearing thresholds, and to identify auditory neurons.

Results: Song encoding was assessed by comparing spike-rate selectivity for conspecific song over heterospecific song to tuning for spectrotemporal modulations. Previous reports found that tuning to spectrotemporal modulations in adult songbirds matched the modulations in conspecific song. We compared neural selectivity for modulations found in zebra or Bengalese finch song, as well as selectivity to modulations not found in either. Results were compared to previous reports of auditory tuning in adult zebra and Bengalese finches to identify changes in tuning over development.

Conclusions: These results suggest that differences in tuning for song acoustics in the secondary auditory cortex may develop before the completion of song learning. Future work will compare tutored and untutored juveniles to identify the contributions of genetic background and experience in the development of auditory tuning for song acoustics.

T138. Neuroplasticity in Older Adults From Auditory-Cognitive Training

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Background: Auditory-cognitive training shows promising behavioral evidence for improving speech-in-noise perception. Previous studies have mainly implemented this training paradigm in hearing-impaired individuals. Even without clinical hearing loss, however, age-related changes in normal-hearing older adults appear to negatively impact speech-in-noise perception, and it is unclear whether these individuals would also receive training benefits. To further assess and validate past behavioral findings, we explored the behavioral and neural effects of auditory-cognitive training in this population.

Methods: In this study, 23 older adults (60-83 years old) underwent 10 sessions of auditory-cognitive training; neural and behavioral measures were collected pre- and post-training. Magnetoencephalography (MEG) was used to record neural responses while participants listened to approximately 60-second audiobook passages under different competing speaker conditions. Applying the temporal response function (TRF) framework, both linear encoding and decoding models were used to analyze neural responses. Self-reported intelligibility and listening effort

were also collected after each passage. Behavioral measures included working memory, speech-in-noise perception, and nonlinguistic auditory stream segregation tasks.

Results: Neural measures include the encoding of acoustic and linguistic features, as well as decoding of acoustic features. We compared the reconstructed stimulus features and TRFs between pre- and post-training sessions and demonstrate training-related neuroplasticity. Moreover, we identified pre-training behavioral measures that may predict training-related neuroplasticity changes.

Conclusions: This study determined the breadth of auditory-cognitive training benefits even in older adults without clinical hearing loss. Our results further support auditory-cognitive training as an intervention to improve listening in noisy environments. Both neurally and behaviorally, these results highlight important measures that may be associated with improved speech-in-noise perception.

T139. Laminar Cortical Dynamics During Perceptual Learning

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Background: Auditory perceptual learning is the process by which training improves the detection, discrimination, or identification of sound stimuli. A deeper understanding of the mechanisms underlying auditory perceptual learning may inform clinical approaches to treating hearing loss and maximizing the benefit of assisted listening devices. Auditory cortical plasticity supports perceptual learning, yet the laminar mechanisms remain poorly defined. Translaminar recordings from the auditory cortex of freely-moving, behaving animals have yet to be described, likely due to technical difficulties associated with the lateral position of the auditory cortex. We developed a chronic translaminar recording approach that overcomes this challenge, and used it to monitor local field potentials in the auditory cortex of freely-moving Mongolian gerbils as they trained on an auditory perceptual learning paradigm.

Methods: Mongolian gerbils were water-restricted and trained on a Go/No-Go aversive paradigm for amplitude modulation (AM) detection. Animals learned to lick during unmodulated broadband noise (NoGo) and withdraw during AM noise (Go) to avoid a mild shock. Once procedural criteria were met, a chronic free-floating 16-channel laminar probe (A1x16-3mm-100-703, Neuronexus) was implanted in the primary auditory cortex and animals began perceptual training. During perceptual training, the AM depth is progressively reduced making detection of the Go stimulus increasingly difficult. Local field potentials were recorded and current source density (CSD) was calculated under three conditions: (1) passive AM exposure before each daily training session, (2) active training, and (3) passive AM exposure immediately after each training session.

Results: Local field potentials were successfully recorded in awake, freely-moving animals over the course of perceptual training. During perceptual training, detection thresholds improved progressively across 10 days, consistent with perceptual learning. Data collection and analysis are still ongoing.

Conclusions: To our knowledge, this work will provide the first laminar characterization of sound evoked activity in awake, freely-moving animals, and will provide new insight into how cortical circuits transform sound representations during perceptual learning.

T140. Disentangling of Spoken Words and Talker Identity in the Human Auditory Cortex

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Background: Complex natural sounds such as speech contain many different sources of information, but recognizing these distinct information sources is computationally challenging because they are highly entangled in the acoustic waveform that reaches the ear. For example, variation in the acoustic attributes of different talkers makes it challenging to recognize the identity of a word, while variation in the acoustics of different words makes it challenging to recognize talker identity. How does the auditory system disentangle word identity from talker identity in auditory cortex? While disentanglement has been extensively studied in the visual cortex, less is known about how it is accomplished in the human auditory cortex. One hypothesis is that specific brain regions specialize for coding either word identity or talker identity. Alternatively, word identity and talker identity might be represented by distinct dimensions of the neural code rather than separated into distinct regions.

Methods: Distinguishing between these two hypotheses has been challenging, in part due to the coarse resolution of non-invasive neuroimaging methods such as fMRI and the difficulty of quantifying invariance from noisy neural signals that never produce the same response twice, even if the underlying response is invariant to acoustics. To address the first challenge, we measured spatiotemporally precise human intracranial recordings to a diverse set of 338 words spoken by 32 different talkers. For the second challenge, we developed novel metrics for quantifying representational disentangling of word and talker identity, both within individual electrodes and brain regions, as well as across different dimensions of the neural population response.

Results: We observed individual electrodes in speech-selective regions of human non-primary auditory cortex that show a representation of words that is partially robust to acoustic variation in talker identity, but no electrodes or brain regions showed a robust representation of talker identity. However, at the population level, we observed distinct dimensions of the neural response that nearly exclusively coded either words or talker identity. Consistent with perceptual data, representations of words were invariant to the presence or absence of pitch information, while representations of talkers were pitch-dependent.

Conclusions: These results suggest that there is partial specialization for talker-robust word identity in localized brain regions, but that robust disentangling is accomplished at the population level with distinct representations of words and talker identity mapped to distinct dimensions of the neural code for speech. Current work, in progress, involves learning multiple dimensions that represent words and talker identity, characterizing the functional and anatomical organization of

these dimensions, and exploring if the uncovered components are tuned to specific features of words or talkers.

T141. Transcriptomic Comparison of Avian Auditory and Vestibular Sensory Epithelia

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Background: The function of the cochlea and vestibule mainly relies on hair cells (HCs), which have similar structural features but different roles in each organ, contributing to hearing and balance. In this study, we computationally compared hair cell transcriptomes from the avian hearing organ, the basilar papilla, and the utricle, a vestibular organ, to examine how these subtypes differ in gene expression within a single species. Our aim is to identify distinct marker genes and transcriptional profiles linked to the physiological functions of different hair cell subtypes.

Methods: We utilized our chicken single-cell RNAseq data of normal basilar papilla and utricle HCs, regenerated basilar papilla HCs, and utricle HCs during turnover. For regenerated basilar papilla HCs, we used data from sensory epithelia 96 hours post-sisomicin treatment. After merging the data, we compared and analyzed them using Seurat. To induce HC regeneration in experiments, we damaged basilar papilla and utricle HCs by administering sisomicin, an aminoglycoside antibiotic, through the lateral semicircular canal. In situ hybridization chain reaction (HCR) was performed to validate the gene expression.

Results: First, we compared the transcriptomic profiles of normal basilar papilla and utricle HCs. Differential gene expression analysis revealed that 348 genes were enriched in basilar papilla HCs, while 163 genes were enriched in utricle HCs. Several marker genes were validated using HCR. Including the transcriptomic data of regenerated basilar papilla HCs, re-analysis showed that regenerated basilar papilla HCs are more similar to extrastriolar type II HCs. HCR confirmed that CALB2, SYN3, RASD2, CRABP1, and NREP were commonly expressed in regenerated basilar papilla as well as utricle extrastriolar type II HCs. Further analysis of the data, including nascent utricle HCs during turnover, both regenerated basilar papilla and nascent utricle HCs indicated similarity to extrastriolar type II HCs. Lastly, we focused on the differences in the regenerative capability of the utricle type I and type II HCs, considering transcriptomic analogy between regenerated basilar papilla HCs and utricle type II HCs. In 3 weeks post-sisomicin administration, regenerated utricle HCs no longer had type I properties, whereas type II HCs regenerated properly.

Conclusions: Our comparative analysis revealed distinct transcriptomic profiles for HCs in the basilar papilla and utricle. A comparison with newly regenerated avian auditory HCs and nascent utricle HCs showed that their transcriptomic features are more similar to utricle type II HCs, than to their homeostatic counterparts. During utricle HC regeneration after damage, type I HCs may transition through type II-like HCs.

T142. Transcriptomic Analysis of Newly Regenerated Avian Auditory Hair Cells

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Background: Inner ear sensory hair cells (HCs) are essential for transducing auditory and vestibular stimuli. In mammals, lost HCs do not regenerate, resulting in permanent sensory deficits. In contrast, non-mammalian vertebrates, such as birds, can regenerate HCs through activation of adjacent supporting cells (SCs). Newly regenerated HCs in the avian hearing organ, the basilar papilla, differ from fully mature HCs by lacking hair bundles and innervation. We hypothesize that the transcriptomic profiles of these nascent HCs are distinct and reflect an intermediate phase of HC identity that precedes full cytomorphological and functional maturation.

Methods: Chicken basilar papilla hair cells were ablated with an injection of sisomicin into the inner ear at post-hatch day 7. We utilized single-cell RNA sequencing to profile the undamaged chicken basilar papilla as well as at 5- and 10-days post-sisomicin treatment (PST). A list of the top marker genes of nascent HCs at different time points was curated. The duration of marker gene expression was determined through qPCR and in situ mRNA detection at various days after the first appearance of nascent HCs at 4 days PST. Ingenuity Pathway Analysis was used to identify signaling pathways activated during nascent HC maturation. To investigate the functional requirement of these pathways, pharmacological inhibitors were infused into sisomicin-treated inner ears during the period of nascent HC maturation.

Results: Single-cell RNA sequencing revealed notable differences in gene expression profiles among undamaged HCs, nascent HCs at 5d PST, and 10d PST. Validation of these distinct marker genes showed that nascent HCs, although formed over several days, exhibit a coordinated onset of a nascent gene expression pattern, followed by a transition toward a gene expression profile indicative of HC maturation. Initial results from signaling pathway inhibition experiments will be presented.

Conclusions: Our findings reveal a unique transcriptomic signature in nascent HCs in the chicken basilar papilla, characterized by the presence of genes not usually expressed by mature HCs. These genes may be required for replacement HC fate commitment and differentiation, and their expression may be specific to regeneration versus normal development during embryonic stages. Understanding the molecular mechanisms of HC regeneration in birds could provide valuable insights for developing reprogramming strategies aimed at inducing HC regeneration in mammals.

T143. Therapeutic Effect and Mechanism of the Contralateral Botulinum Toxin Injection for Facial Nerve Palsy in Rat Model

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Background: Facial palsy (FP) leads to significant functional, aesthetic, and psychosocial impairment. Current treatments aim to maximize recovery while minimizing the duration of disability. Botulinum toxin (BTX) injection into the contralateral facial muscles has been applied clinically as an adjunct therapy, but its underlying mechanism for promoting faster recovery

remains unclear. This study aimed to investigate the therapeutic effects and possible mechanisms of contralateral BTX injection in a rat model of peripheral FP.

Methods: Male Sprague–Dawley rats (n=3 groups) were assigned to: control (CG), facial palsy without treatment (FP), and facial palsy with contralateral BTX injection (FPB). FP was induced by crushing the main trunk of the facial nerve near the stylomastoid foramen for 1 minute under microscopy. In FPB, 20 mouse units of BTX-A were injected at three points of the contralateral facial muscles 1 day after FP induction. Functional recovery was assessed by vibrissae movement scale and nasal deviation angle at 3 days, 1, 2, and 4 weeks post-injury. Histological and molecular analyses included H and E staining of the crush site, immunofluorescence (IF) of neuromuscular junctions (synaptophysin and α -bungarotoxin), Western blot of neuropeptides (PACAP, galanin, CGRP) in the brainstem, and IF for c-Fos in the motor cortex.

Results: FP was successfully induced in all animals. In vibrissae movement analysis, FP and FPB scores were significantly reduced versus CG at early time points; however, by week 2, FPB recovered to CG levels, while FP remained significantly impaired. Nasal deviation was significantly greater in FP than CG at all time points, whereas FPB showed no significant deviation compared to CG from day 3 onward. H and E staining revealed earlier restoration of nerve continuity and fiber density in FPB (by week 2) compared to FP (week 4). NMJ IF showed significantly reduced synaptophysin intensity in FPB versus CG at day 3, but no intergroup differences thereafter; α -bungarotoxin intensity remained reduced in FP and FPB until week 2, normalizing by week 4. PACAP expression in the brainstem was significantly lower in FPB at week 2 compared to CG and FP; CGRP and galanin showed no significant group differences. Motor cortex IF demonstrated significantly higher c-Fos-positive cell counts in FPB at day 3 compared to CG and FP, with both FP and FPB elevated versus CG at weeks 1 and 2, normalizing by week 4.

Conclusions: Contralateral BTX injection accelerated functional and morphological recovery after facial nerve crush injury in rats, with earlier restoration of facial symmetry, nerve continuity, and NMJ integrity. Changes in brainstem neuropeptide levels and cortical activation patterns suggest central as well as peripheral mechanisms may contribute to recovery. These findings support contralateral BTX as a potential adjunctive strategy for enhancing FP rehabilitation, warranting further mechanistic and translational studies.

T144. Camp and Pka Signaling is Essential for Mitotic Hair Cell Regeneration in the Basilar Papilla in Vivo

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Background: Hearing loss is a global health issue with a profound impact on quality of life and currently has no restorative treatments. Mammals suffer from permanent hearing loss due to an inability to regenerate sensory hair cells. In contrast, non-mammalian vertebrates, like birds, regenerate hair cells and restore hearing throughout life, a process driven by supporting cell

proliferation. Our prior work identified an essential signaling pathway for supporting cell S-phase entry and hair cell regeneration in vivo after hair cell damage induced by an ototoxic drug, sisomicin. To deepen our understanding, we now explore additional pathways that regulate mitotic quiescence in the undamaged basilar papilla and promote supporting cell proliferation after hair cell ablation. Previous in vitro studies found that cyclic AMP (cAMP) activators stimulate proliferation in undamaged auditory sensory epithelium, a response blocked by cAMP-regulated protein kinase A (PKA) inhibitors. Our study uses a robust in vivo model to validate these findings.

Methods: In post-hatch day 7 (P7) chickens, cAMP levels were pharmacologically elevated in the damaged basilar papilla by co-infusing cAMP activators with the ototoxic sisomicin into the lateral semicircular canal. cAMP activators were also infused into the undamaged basilar papilla. Supporting cell proliferation was quantified via EdU incorporation 48 hours post-infusion. To assess the role of PKA, we infused PKA inhibitors with sisomicin and measured their effect on supporting cell proliferation.

Results: Elevating cAMP levels with 8-Bromo-cAMP and 3-isobutyl-1-methylxanthine (IBMX) in the damaged basilar papilla resulted in a significant increase in supporting cell proliferation compared to sisomicin treatment alone. Conversely, PKA inhibitors blocked the typical proliferative response in supporting cells observed after sisomicin treatment. Surprisingly, in the undamaged basilar papilla, co-administration of cAMP activators caused only a minimal increase in supporting cell proliferation, as indicated by sparse EdU incorporation.

Conclusions: Our results reveal that the cAMP/PKA signaling pathway is essential for triggering supporting cell proliferation in vivo after damage. Furthermore, while this pathway can modestly promote S-phase entry in the absence of damage, it is primarily an essential component of the proliferative response to injury. Together with our previous work, these findings demonstrate that multiple intracellular signaling pathways are necessary for S-phase entry of basilar papilla supporting cells in response to hair cell loss. Future research must investigate how these distinct pathways interact to coordinate a precise regenerative response that effectively restores auditory function in the avian inner ear. These insights enhance our understanding of sensory hair cell regeneration in birds and lay the groundwork for future translational studies for hearing loss in mammals that utilize signal transduction pathways as potential therapeutic targets.

T145. Lats Kinase Inhibition Induces Cell Cycle Re-Entry of Postmitotic Supporting Cells in Mouse Inner Ear Organoids via Yap Nuclear Translocation

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Background: Loss of sensory hair cells (HCs) in the mammalian inner ear leads to permanent hearing and balance dysfunction. In contrast to non-mammalian species, adult mammalian supporting cells (SCs) are quiescent and cannot mitotically regenerate mature HCs. Using mouse embryonic stem cell (mESC)-derived inner ear organoids (IEOs), we previously observed that SCs exit the cell cycle between day in vitro (DIV) 16 and DIV21, mirroring the cell cycle exit in the mammalian inner ear. Based on this finding, we propose using IEOs as a scalable and accessible alternative to animals for studying cell cycle exit and as a drug development platform

for SC cell cycle re-entry to replenish lost HCs. As a proof-of-concept, we tested the feasibility of inducing cell cycle re-entry in postmitotic sensory epithelia of late-stage IEOs using the LATS kinase inhibitor TRULI, which has been previously shown to promote proliferation of postmitotic SCs in vivo through YAP nuclear translocation.

Methods: To characterize SC cell cycle exit, we quantified proliferation marker Ki67-positive otic cells daily between DIV16 and DIV24. To capture the complete S-phase entry dynamics, we then exposed IEOs to 10 μ M EdU in 24h increments over the same period and quantified EdU+ otic cells. Three biological replicates (independent IEO batches) and five technical replicates (transections) were analyzed at each time point. Based on the delineated timeline, we identified the time point when mitotic quiescence was achieved and applied 10 μ M TRULI treatment for 72h. SC proliferation was evaluated via Ki67 expression or EdU incorporation. To assess the mechanism through which TRULI took effect, we analyzed YAP localization by calculating the nuclear-to-cytoplasmic ratio of YAP expression with and without TRULI exposure.

Results: SCs showed a gradual decline in proliferation from DIV16 to DIV24, with near complete quiescence by DIV23, which was selected as the starting point for TRULI treatment. After 72h exposure to 10 μ M TRULI, DIV26 SCs displayed a significant increase in proliferation, as indicated by a higher percentage of EdU+ otic cells. TRULI treatment also increased the nuclear-to-cytoplasmic YAP ratio, suggesting that proliferation was mediated by YAP nuclear translocation, consistent with its previously described in vivo mechanism of action.

Conclusions: This proof-of-concept experiment demonstrates that LATS kinase inhibition induces cell cycle re-entry of postmitotic SCs in late-stage IEOs by facilitating YAP nuclear translocation. These findings highlight the value of mouse IEOs as a scalable and accessible alternative to animal models for studying cell cycle exit in the mammalian inner ear, as well as an in vitro platform for developing strategies to induce SC proliferation for HC regeneration. Furthermore, our quantitative assessments validate the potential of integrating genetic tools such as CRISPRa and CRISPRi into IEOs to establish a high-throughput platform for discovering pro-regenerative therapies for inner ear disorders.

T146. Three-Dimensional Spheroid Cultures as a Model for Chemoresistance in Head and Neck Cancer

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Background: Head and neck cancer remains one of the most refractory malignancies, and treatment failure is often associated with the development of chemoresistance. Understanding the mechanisms underlying this resistance is therefore crucial for improving therapeutic outcomes. In vitro, three-dimensional (3D) culture systems have recently gained attention as physiologically relevant models that more closely mimic the tumor microenvironment compared with conventional two-dimensional (2D) monolayer cultures. In this study, we investigated the differences in chemoresistance between 2D and 3D cultures of head and neck cancer cells and analyzed the associated changes in epithelial–mesenchymal transition (EMT) marker expression.

Methods: The head and neck squamous cell carcinoma cell lines SAS and HSC-3 were seeded at 3.1×10^5 /mL. For 2D culture, cells were grown in 48-well flat-bottom plates. For 3D culture, spheroids were generated using concave culture vessels (CuPS). Two conditions were examined:

spheroids maintained continuously in CuPS and spheroids transferred onto 2D plates after initial formation. Anticancer drugs used were cisplatin (CDDP), 5-fluorouracil (5-FU), and their combination (CDDP+5-FU), administered one day after seeding. Drug sensitivity was evaluated using MTT and ATP assays, with IC₅₀ and IC₈₀ values calculated to compare responses. EMT-related changes were assessed by monitoring cadherin and vimentin expression as representative markers.

Results: Compared with 2D monolayers, 3D spheroid cultures exhibited significantly greater resistance to anticancer drugs. The degree of resistance varied depending on the drug regimen, and EMT marker alterations differed among treatments. Spheroid cultures maintained in CuPS displayed stable morphology and enhanced drug tolerance, whereas spheroids transferred to 2D plates tended to disintegrate, leading to distinct morphological responses under drug exposure. Differences in EMT marker expression between these culture conditions further indicated that the structural and environmental context of the spheroids strongly influenced cellular behavior. Importantly, even when identical cell lines and drug treatments were applied, resistance patterns and EMT changes diverged according to the microenvironment.

Conclusions: Our findings demonstrate that 3D spheroid cultures more accurately reflect the complexity of the in vivo tumor microenvironment by exhibiting enhanced chemoresistance and dynamic alterations in EMT markers. The results suggest that microenvironmental context plays a pivotal role in shaping drug responses and may underlie fundamental mechanisms of resistance in head and neck cancer. Thus, 3D spheroid models represent not only a useful tool for experimental drug testing but also a promising platform for uncovering the biological basis of chemoresistance and guiding the development of more effective therapeutic strategies.

T147. A Search for Correlates of Modulation Masking in Electrophysiological Envelope Following Responses From Younger and Older Adults

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Background: Modulation masking is known to play an important role in the perception of speech in fluctuating acoustic backgrounds. Recent studies have shown broader tuning of modulation masking in older compared to younger adults with normal hearing. Studies using electroencephalographic (EEG) measures of neural responses synchronized to temporal envelopes have reported stronger speech envelope tracking in older than younger adults. Both perceptual and neural effects have been attributed to loss of age-related neural inhibition. However, a direct link between modulation masking and EEG-based measures of neural envelope tracking has not been established.

Methods: EEG recordings were performed in younger (18 – 30 yrs) and older (55 – 70 yrs) adults to obtain envelope following responses (EFRs) to amplitude-modulated (AM) noise bursts while the listeners watched silent (captioned) movies. In separate experimental runs, the noise was modulated by a single sinusoidal, ramped (slow onset, fast offset), or damped (fast onset, slow offset) AM waveform, while some runs also included a second sinusoidal AM of a different rate. AM rates were selected to measure neural activity dominated by cortical (8 and 11 Hz), thalamic-cortical (41 and 51 Hz), and subcortical (102 Hz) generators. We hypothesized that the

‘target’ EFRs in the presence of the second AM rate will be suppressed compared with that for the target rate presented alone.

Results: EFRs derived from the EEG waveforms did not show patterns consistent with perceptual modulation masking, i.e., EFRs for single-rate AM were not consistently suppressed by another AM. This was true for low (8 and 11 Hz) and medium (41 and 51 Hz) modulation rates. Adding a spectrally remote 102-Hz AM appeared to have no effect on the EFR for the 8-Hz AM and suppress the EFRs for 41- and 51-Hz AM. Older listeners had stronger EFRs for the 8-Hz AM but tended to have weaker EFRs for the higher AM rates than younger listeners. EFRs for the ramped and damped envelopes of the same rate were similar and generally weaker than those for sinusoidal AM, but they were differentially affected by simultaneous sinusoidal AM with a proximal rate.

Conclusions: In conclusion, no correlate of modulation masking was systematically reflected in EFRs for the AM rates and envelope shapes used, indicating that perceptual modulation masking was not reflected in EEG-based measures of synchronized neural activity at cortical and thalamic-cortical sites. The stronger 8-Hz EFRs observed in older listeners cannot be explained in terms of greater reliance on cognitive resources as listeners were not actively performing any task involving the modulated stimuli. Future studies will focus on a metric derived from cortical auditory event-related potentials to capture global responses from neurons that encode AM by synchrony and average firing rates. [Supported by NIH R01 DC 021362 (Wojtczak)].

T148. Emotional Learning Induced Changes to Human Auditory Processing

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Background: Perceptual learning drives experience-dependent plasticity in the brain, enabling faster and more efficient processing of sensory stimuli. Animal models have empirically shown that neuroplastic changes in sensory systems are amplified when perceptual learning is paired with emotionally valent conditions (e.g., fear of electrical shocks), leading to long-lasting alterations in how stimuli are encoded at both cortical and subcortical levels. The extent to which emotional learning influences cortical and subcortical sensory processing in the human brain remains poorly understood but is a key factor in developing models, objective measurements, and treatments for sound tolerance disorders. The goals of this ongoing study are to objectively measure and define the time course of cortical and brainstem neuroplastic changes associated with Pavlovian fear conditioning paradigm.

Methods: Normal hearing listeners continue to be recruited for this ongoing study. Participants undergo a three-phase test protocol (Baseline, Conditioning, and Extinction) during which auditory evoked cortical and brainstem responses are collected with a 64-channel EEG cap in addition to skin conductance responses (SCR). During the Baseline phase, two complex tones are presented to the listener. In the Conditioning phase, one complex tone (CS+) is paired with an electrical shock delivered to the wrist on 50% of trials, whereas the other tone remains benign (CS-). In the Extinction phase, CS+ and CS- tones are presented without electrical shock.

Neural data are filtered to extract brainstem (70-1000 Hz) and cortical (1-30 Hz) evoked potentials in response to auditory stimuli. Cortical and brainstem response amplitudes and topographies will be compared across the three phases of the experiment to examine fear-

induced neuroplastic changes. Additionally, an endogenous potential associated with anticipation of an emotionally-valent stimulus (the Stimulus Preceding Negativity; SPN) will be measured from the Conditioning and Extinction phases. This potential has been linked to emotional learning.

Results: Preliminary results indicate that the cortical onset P1-N1-P2 responses to CS+ stimuli are enhanced relative to Baseline after fear conditioning. Additionally, SPN has been observed in the Conditioning phase in most participants, suggesting its use as an objective measure of auditory learning.

Conclusions: Future work will include listeners with sound tolerance disorders (e.g., misophonia) to determine if fear-based auditory learning differs in this group compared to typically functioning controls.

T149. Dynamics of Switching Attention in a Cocktail Party Problem

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Background: Neural representation of the auditory cortex has been well shown to represent the attended conversation more strongly than the unattended one in a cocktail party situation. In other words, a high level cognitive process in humans is picking out the attended talker out of the complex scene to give the person the ability of focusing and therefore better understanding of the conversation within the noisy environment. What remains unclear is the cognitive process driving the change of the neural representation in the auditory cortex. Leveraging intracranial EEG (iEEG) brain recording while changing attention throughout the trials makes it possible to have a better understanding of the cognitive network behind this process, and timing properties of the attention switches.

Methods: As explained, the aim is to reveal cortical areas and timing features involved in attention switches. To identify brain regions engaged in this process, we applied statistical analyses to the activity of the recorded regions during the attention build up of the new source. To further characterize timing dynamics of the attention switch, a projection of brain activity to a latent space was used allowing us to track different processing states over time.

Results: For this study, 5 subjects having drug-resistant epilepsy were implanted with subdural ECoG grids and sEEG electrodes. An auditory scene of two simultaneous conversations were presented to the subjects by two speakers positioned at the front right and front left. An arrow on the screen indicates which conversation the subject should attend to. We considered different frequency bands and the results suggest regions beyond auditory regions are playing roles in switching of attention while not representing acoustic features like talker gender. Observations show multiple stages the brain goes through to redirect attention over a time scale of a few seconds.

Conclusions: In everyday listening, people often shift their attention from one source of audio to another one within complex auditory scenes. While previous studies have shown how the auditory cortex enhances the representation of the currently attended source, less is known about what exactly is driving this effect. This study provides evidence of large scale cortical networks contributing to attention switching in auditory scenes. It highlights the temporal dynamics of this

process. Findings of this study helps how attention shifts are coordinated in the human brain in auditory scenes.

T150. Thalamo-Cortical Connectivity Strengthens in Patients With Single-Sided Deafness; Resting-State fMRI Study

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Background: Strengthened ipsilateral auditory pathways have been reported as a typical neuroplastic phenomenon in patients with single-sided deafness (SSD). In the auditory cortex contralateral to the deaf ear, responsiveness to stimulation of the intact ear has been shown to increase and has been associated with behavioral outcomes. However, evidence remains limited regarding how this occurs and whether it differs according to the side of deafness. This study leveraged a high-resolution parcellation of the auditory cortex, enabling seed-based resting-state connectivity analyses to be performed separately for each of the three cytoarchitectonically defined subregions in SSD patients.

Methods: Nineteen left- and 18 right-sided SSD patients (LSSD and RSSD, onset age > 15 years) and 28 normal-hearing controls (NC) participated; all were right-handed and had no history of neurological or psychiatric illness. Resting-state fMRI data (100 volumes) were acquired on a 3T Siemens Skyra using a gradient echo–planar sequence (TR = 3000 ms, TE = 30 ms), together with T1-weighted anatomical images. Seed-based connectivity analyses were performed using the CONN toolbox within MATLAB. Six auditory subregions (Te1, Te2, and Te3 in each hemisphere) served as seeds. Group-level analyses employed a general linear model, with clusters reported at voxel-level $p < 0.001$ (uncorrected) and cluster-level $p < 0.05$ (FWE-corrected).

Results: Across all six auditory seeds, NC and SSD groups showed significant autocorrelation within each seed and connectivity with contralateral homologous regions and the cingulate cortex. In NC, thalamo-cortical connectivity was significant only between the right Te1 and the right thalamus, whereas both SSD groups exhibited multiple significant connections of Te1 and Te2 seeds with the thalamus bilaterally. Importantly, the Te2 seed contralateral to the deaf ear showed connectivity with the thalamus contralateral to the intact ear. NC also demonstrated connectivity between both Te2 seeds and the calcarine cortex, a pattern observed only for the left Te2 seed in RSSD. Moreover, in RSSD, all three right-hemisphere auditory subregions revealed significantly increased connectivity compared with NC.

Conclusions: In SSD, thalamo-cortical connectivity along both ipsi- and contralateral pathways is enhanced, supporting a strengthened bottom-up drive from the intact ear. Functional auditory connectivity is particularly increased in RSSD compared with NC. These findings suggest compensatory reorganization of auditory networks, with potential implications for rehabilitation strategies in SSD.

T151. Oligodendrocytes Proliferation in the Auditory Brainstem of Fragile X Syndrome Mice

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Background: The auditory brainstem, specifically the medial nucleus of the trapezoid body (MNTB), possesses heavily myelinated neurons to aid in the rapid processing of information. The myelin sheaths are formed by the oligodendrocyte glial cells, making them crucial to the processing of information, including sound information. Studies have shown that deficits in myelination contribute to autism spectrum disorder (such as Fragile X syndrome) phenotypes. Thus, suggesting that brain areas that depend on the rapid processing of information, like the auditory brainstem, can be affected. A previous study by Lucas et al. showed an increase in both oligodendrocytes (OLs) and their precursor cells (OPCs) in the auditory brainstem, which may be related to a deficit in myelination and the function of OLs and overcompensation by OPCs.

Methods: Twelve animals, six of each genotype (FXS and B6 control) under a hundred days old were used. We used the EdU cell proliferation assay to monitor DNA synthesis and cell proliferation. We used antibodies for OLs and OPCs to distinguish EdU incorporation in the two oligodendrocyte cell types.

Results: Results are still in progress. Using a confocal microscope to quantify OLs and OPCs in the auditory brainstem of Fragile X syndrome mice, we expect to see an increase in OLs and OPCs with EdU, suggesting higher turnover of these populations.

Conclusions: Impairments in oligodendrocytes can disrupt the proper myelination of neurons. Understanding how these impairments relate to the most common sensory phenotype observed in Fragile X syndrome (FXS), auditory hypersensitivity, can improve treatment approaches for this neurodevelopmental condition. Given that auditory problems are among the most prevalent symptoms in FXS patients, this study is particularly relevant to their symptomatology. Future research on how an imbalance of oligodendrocytes (OLs) and oligodendrocyte precursor cells (OPCs) affects sound processing may further enhance treatment options for individuals with FXS.

T152. A Model of Speech Recognition Reproduces Signatures of Human Speech Perception and Reveals Mechanisms of Contextual Integration

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Background: Humans rapidly and dexterously transform acoustic waveforms into linguistic representations despite the inherent variability of the speech signal. The mechanisms that enable such robustness remain poorly understood, in part due to the lack of stimulus-computable models that both approach human performance and can test theoretical principles and mechanistic hypotheses.

Methods: We built an artificial neural network model of continuous speech recognition optimized to produce sequences of sublexical units (phonemes or characters) from simulated cochlear representations. The front end simulated outer, middle, and inner ear filtering, followed

by half-wave rectification and low-pass filtering at 4 kHz to approximate the upper limit of mammalian auditory-nerve phase-locking, yielding cochleagrams that served as input to stacked convolutional and recurrent layers trained to predict sublexical sequences. The model was trained on 15,000 hours of speech data superimposed on naturalistic noisy backgrounds, with varied SNRs and sound levels.

We compared the model to humans using a new large-scale benchmark of nonword transcription and discrimination along with a battery of previously documented signatures of speech perception, including categorical perception, neighbourhood density effects, phonotactic probability effects, formant-based vowel space, and benefits of talker familiarity. We tested the role and importance of contextual integration by ablating contextual integration mechanisms (recurrent processing), and by manipulating the directionality of contextual processing in the model.

Results: Human nonword transcription and discrimination exhibited highly reliable phoneme confusion structure (split-half $r=0.98$). The model closely matched human performance, reproducing human-like patterns of phoneme recognizability ($r=0.96$) and confusions ($r=0.95$). It also recapitulated classic human perceptual signatures: categorical perception of consonants, sensitivity to neighborhood density and corpus statistics, formant-based vowel encoding, and improved recognition with talker consistency.

Removing contextual processing substantially reduced human–model similarity, suggesting that human recognition depends critically on integrating surrounding speech information.

Conclusions: We built a biologically grounded stimulus-computable model of speech perception, and developed a suite of evaluations with which to compare it to humans and probe the basis of recognition. The model aligns closely with human behavior on speech perception tasks, but only if allowed to integrate information from the surrounding speech signal, implicating contextual processing in human speech perception. By providing both a working model and an evaluation suite, this work supports systematic assessment of existing and future theories of speech perception.

T153. Older Adults Show Overexaggerated and Larger Noise-Related Degradation in Their Neural Entrainment to Speech

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Background: Speech-in-noise (SIN) perception is a difficult everyday listening task that becomes even more difficult with age. Neural entrainment, or the yoking of internal oscillations to external stimuli such as speech, is associated with successful speech perception across listening environments; however, how aging impacts neural entrainment to SIN and relates to behavioral decrements in older adults' SIN perception remains unclear. To address these questions, we measured neural entrainment during a rapid SIN perception task in younger and older adults via multichannel EEG.

Methods: Participants ($n=83$) monitored a continuous stream of syllables (~ 4.5 Hz) presented in quiet and noise conditions and indicated the presence of infrequent oddball syllables via button press. We assessed EEG-derived neural phase-locking value (PLV) to the target speech stream

across the scalp to investigate interactions between aging and noise on neural entrainment to speech.

Results: Compared to younger adults, older adults demonstrated higher overall PLV despite poorer behavioral performance. Older adults also showed greater neural and behavioral degradations in speech processing between clean and noise conditions relative to younger listeners. Greater noise-related reductions in behavioral accuracy during lab-based tasks correlated with poorer SIN scores on standardized tests (QuickSIN). Neurophysiological responses (PLV) did not correlate with behavior. However, age remained a strong predictor of neural and behavioral responses to speech even after controlling for hearing loss.

Conclusions: Older adults demonstrated overexaggerated neural entrainment to ongoing speech presented in quiet than younger adults and greater noise-related reductions in speech processing as measured by neural entrainment and perception. Our results demonstrate unusually large speech envelope encoding in older listeners and suggest more robust neural entrainment to the speech signal is not always perceptually advantageous. Though both age and hearing loss are associated with overexaggerated neural entrainment to speech at frequencies relevant for naturalistic speech, age accounts for speech deficits beyond the effects of hearing loss. The lack of correspondence between behavior and neural PLV suggests SIN perception may rely on a more complex set of neural mechanisms than neuro-acoustic synchronization alone.

T154. Examining Facilitation of Speech-In-Noise Perception Through Auditory Rhythmic Cues

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Background: Difficulties with speech-in-noise (SIN) perception are a common audiologic complaint, and yet behavioral interventions are lacking. Previous research demonstrates that periodic, isochronous auditory rhythmic cues matched to rate of target speech facilitate SIN perception. However, it is unknown whether periodicity or predictability (i.e., cue timing matched to the speech envelope) plays a stronger role in SIN facilitation. Additionally, it is unclear how varying levels of background noise influence the perceptual SIN benefit from concordant rhythmic cues.

Methods: Young, normal-hearing adult participants completed a novel, rhythmically-cued SIN perception task by identifying color-number combinations in Coordinate Response Measure sentences. Sentences were presented amidst varying levels of noise (-2.5 dB, -5 dB SNR) and were preceded by auditory rhythmic cues uniquely tailored to each target sentence. Rhythmic cues were either periodic (metronome-like cues at each sentence's average syllable rate), syllable-matched (based on the timing of the exact target sentence syllable nuclei), random (control), or absent (used to determine baseline performance). Participants were instructed to utilize the rhythms to assist target sentence recognition. Accuracy (percent correct) and reaction time were recorded. Participants also completed external measures of musical beat perception (Beat Drop Alignment Test), SIN perception (QuickSIN), and auditory working memory (Digit Span).

Results: Preliminary results demonstrate greater improvements in accuracy and reaction time for periodic rhythmic cues than random cues relative to the baseline condition (no auditory cue),

with greater facilitation at more difficult SNRs. While both experimental forms of rhythmic cueing were effective in bolstering SIN perception to some degree, periodic cues elicited greater improvements than syllable-matched cues. Faster reaction times (but not accuracy) correlated with higher auditory working memory scores. Lab-based tasks and external measures were not correlated.

Conclusions: Our results suggest a beneficial facilitation of SIN perception following periodic auditory cues, especially in more difficult noise-degraded listening conditions. Greater benefit from periodic than syllable-matched cues could indicate a stronger role of periodicity (rather than predictability alone) in perceptual tracking and subsequent decoding of the speech signal. We discuss implications for neural and behavioral mechanisms of rhythmic SIN cueing benefits.

T155. An Investigation of the Relationship Between Speech Recognition in Noise and Temporal Resolution Index for Listeners With Normal Pure-Tone Average: Focusing on Modulation Detection Thresholds

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Background: Some individuals with clinically normal audiometric thresholds experience difficulty in understanding speech in noise, i.e., hidden hearing loss. Auditory temporal resolution declines even in individuals with normal audiometric thresholds. Reduced temporal resolution impairs the ability to accurately perceive the amplitude envelope of speech and noise signals. In quiet environments, speech intelligibility relies on the perception of slow temporal modulations (4–8 Hz) that convey syllabic rhythms and essential envelope cues. In speech recognition in noise, the ability to perceive high temporal modulation (> 8 Hz) is important because high temporal modulation may serve as a cue for separating noise from speech. This study examined the relationship between the modulation detection threshold (MDT) at various modulation frequencies and speech intelligibility in noise to identify the critical modulation frequency range for understanding speech in noise.

Methods: We measured the speech reception threshold (SRT) in noise and MDTs at modulation frequencies of 8, 16, 64, 128, and 256 Hz in 58 young and 29 elderly adults, with a normal pure-tone average. SRT in noise was measured by the Noise-Front condition of the Japanese-HINT (hearing in noise test), in which both speech and noise signals were presented from the speaker directly in front of the listener. To evaluate the relationship between SRT in noise and MDT at each frequency, we calculated partial correlation coefficients while controlling for participants' age because age is often correlated with SRT and MDT, which might result in pseudo-correlations between SRT and MDT.

Results: The obtained partial correlation coefficients between SRT and MDT were: $r=0.103$, $p=0.346$ for MDT at 8 Hz; $r=0.152$, $p=0.163$ for MDT at 16 Hz; $r=0.183$, $p=0.091$ for MDT at 64 Hz; $r=-0.001$, $p=0.991$ for MDT at 128 Hz; $r=0.210$, $p=0.053$ for MDT at 256 Hz.

Conclusions: A significant, although marginal, correlation was obtained only between the SRT in noise and the 256 Hz MDT. The correlations at lower modulation frequencies were not significant. These results support our hypothesis that the perception of high modulation frequencies is important in separating speech from noise.

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T156. Consonant and Vowel Cue Transmission in Cochlear Implant Users: Prelingually-Implanted Children Versus Post-Lingually Implanted Adults

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Background: While many early-implanted children with cochlear implants (CIs) achieve good speech understanding in quiet, outcomes remain highly variable and difficult to predict. Children with and without hearing loss struggle more than adults to understand speech in noise, suggesting ongoing development in how speech cues are processed. Typical clinical speech perception measures word or sentence recognition, which does not provide a detailed assessment of how a listener may be using phonetic features of speech. Therefore, our lab is interested in evaluating specific acoustic cues of vowels and consonants, as this may help better identify how speech factors, such as temporal or spectral cues, are correlated to speech perception, and why this appears different in children and adults. Previously, our lab investigated the relationship between spectral resolution, a known predictor of speech perception in CI users, and consonant and vowel cues. These findings suggested developmental differences between children and adults, especially in noise. This study builds on previous work by examining age-related differences in acoustic cue transmission for consonants and vowels.

Methods: Participants included 11 post-lingually implanted adults (56-83yo) and 14 children (recruited at 5-10 years) implanted prior to age 2. Children are tested annually for three years in an ongoing longitudinal study. All testing was completed with their preferred CI processor and the contralateral ear unaided/plugged if necessary. Consonant and vowel identification were measured using a closed set /a/-consonant-/a/ and /h/-vowel-/d/ task spoken by a male talker. Testing was completed in 60 dBA in quiet (with a subset of listeners tested in +10 dB SNR multi-talker babble) scored by percent correct, which also provided a matrix of error patterns. Error patterns were systematically analyzed using the sequential information analysis (SINFA) for consonants (Voicing, Manner, Place) and vowels (Duration, F1, F2). SINFA scores were analyzed with separate linear mixed-effects models per cue, including fixed effects of Age, Condition, and their interaction, plus a random intercept for Subject. Effects were tested via Type-III Wald χ^2 , and Quiet vs. Noise differences were interpreted using estimated marginal means (EMMs).

Results: For consonant cues, both Manner and Place were significantly degraded by background noise across all age groups. Voicing cues showed a significant Age x Condition interaction: younger children (5–7yo) demonstrated a pronounced noise-related deficit that diminished with age. For vowel cues, trends suggested reduced accuracy in noise for F1 and Duration, though effects were less robust than for consonant cues. Marginal Age × Condition interactions for F2 and Duration indicate possible developmental changes in how vowel cues are processed in noise.

Conclusions: These findings highlight the disproportionate impact of noise on speech cue perception in young children, particularly for voicing contrasts. Future work will examine the relationship between acoustic cue transmission and spectral and temporal modulation perception.

T157. Cortical Processing of Audiovisual Speech With Naturalistic and Avatar-Based Visual Input Under Background Noise: Evidence From Meg

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Background: Understanding speech in acoustically challenging environments often requires more than auditory input alone. Visual cues, such as a speaker's lip movements, can enhance comprehension. Recent studies show that facial animations generated from speech can similarly facilitate understanding, offering a promising tool to aid individuals with hearing difficulties. However, the cortical mechanisms underlying audiovisual (AV) speech perception with such avatars remain unclear. Here, we investigated how different types of visual input accompanying speech modulate auditory cortical activity under noisy listening conditions, with a focus on temporal response functions (TRFs).

Methods: We recorded magnetoencephalographic (MEG) responses from 32 normal-hearing participants while they listened to short five-word sentences from the Oldenburger Satztest. Sentences were presented against a continuous four-talker babble noise derived from audiobooks, creating a realistic adverse listening environment. Each trial was shown under seven visual conditions: four audiovisual (still image, degenerated video, avatar, and natural video of the speaker) and three visual-only conditions. After each sentence, participants repeated every word they understood, enabling precise behavioral scoring. TRFs were computed using multiple speech features: low delta (0.5–1 Hz), delta (1–4 Hz), theta (4–8 Hz), broadband (1–20 Hz), envelope onset (1–20 Hz), and word onset.

Results: Behavioral performance showed robust, significant differences across visual conditions ($p < 0.001$), with comprehension improving progressively from still image to edge detection, avatar, and the natural video. TRF reconstruction scores correlated strongly with behavioral data across conditions ($p < 0.001$), confirming that speech entrainment in auditory cortex reflects comprehension even in noise. Significant differences emerged between audio-only and audiovisual conditions, demonstrating a clear cortical benefit of visual input. Interestingly, for the low delta band, reconstruction scores were significantly reduced in the avatar condition compared to all other audio and audiovisual conditions ($p < 0.001$). This suggests that while avatars support comprehension behaviorally, they may also introduce atypical or conflicting visual information, particularly affecting cortical tracking of slow temporal fluctuations associated with lip-reading.

Conclusions: Our results demonstrate that under realistic background noise, avatars can enhance behavioral speech comprehension, similar to natural videos of a speaker. This benefit is mirrored in auditory cortical entrainment, as revealed by TRFs. However, reduced low delta tracking in the avatar condition suggests that cortical mechanisms of audiovisual integration may be sensitive to subtle mismatches between visual and auditory signals. These findings highlight both the potential and the current limitations of avatar-based communication support, especially in noisy everyday environments.

T158. Utilizing a Head Trauma Index Assessment to Predict Speech Understanding in Noise in a Concussion Population

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Background: Mild traumatic brain injury (mTBI) has been associated with many different cognitive and perceptual difficulties, including understanding speech in noise, despite normal hearing thresholds. Research has characterized the relationship between executive functions and speech perception in a normal hearing population with no history of mTBI. This relationship was not observed when evaluating mTBI as a categorical variable. The current study explores how the number of injuries, and the recency of reported injuries, factors into the model. It was predicted that assessing mTBI as a continuous predictor will better characterize the cognitive and perceptual behavioral outcomes of the mTBI population.

Methods: Participants (n=177) were drawn from two parent studies and self-reported details about their mTBI history. Participants completed a full hearing test, multiple frequency modulation detection tasks, and cognitive and speech in noise assessments.

To evaluate the severity of the mTBI, a continuous scale called the Head Trauma Index (HTI) was calculated. The HTI takes the square root of the number of reported events (E), multiplied by the recency score (R). R is calculated by taking the natural logarithm of the reciprocal of the number of days since most recent mTBI. These two values are then multiplied to get the HTI.

Speech understanding in noise was measured through an iPad task using a virtual spatial array where three utterances were either presented at the same spatial location or different locations, where the maskers were located 45 degrees to the left and right of the listener. Participants were instructed to respond to the target speaker and ignore the other two speakers. Target to masker (TMR) ratios were calculated from the separated and colocated condition on this task by subtracting the target level from the masking level (dB). The difference between the two TMR's was taken to get the spatial release from masking (SRM) value.

Results: A higher HTI score was associated with poorer performance on the separated condition on the speech in noise task, when using an HTI score cutoff of 0.40, which equates to a cutoff of around 7 years from day of testing. Participants who had one or more injuries more than 7 years prior were more likely to have results like those of the control group.

Conclusions: Here we found that the HTI, a continuous injury-load metric, captured individual differences that binary grouping missed, especially for spatial speech in-noise performance. The HTI can be used to better distinguish the mTBI patient population, better assess how different areas of cognition and perception may be compromised by brain injury, and how the number of injuries and recency of injury may play a role in post-injury symptoms.

T159. Investigating the Neural Oscillatory Features for Limited Access to Oral Language in Deaf Children With Early Bilateral Cochlear Implantation

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Background: Cochlear implants (CIs) enable access to auditory input for children with congenital severe-to-profound hearing loss and are most effective when implanted early, typically before the age of 2. However, substantial individual variability in language development remains. The underlying neural mechanisms of this variability remain poorly understood. One promising line of investigation focuses on neural oscillations, particularly the coupling between theta (4–7 Hz) and low- and high-gamma (25–35 Hz and > 40 Hz) frequency bands during speech perception, which are known to support speech phonemic representations and the hierarchical processing of speech. A particular focus is placed on children with GJB2 mutations, the most common genetic cause of non-syndromic congenital deafness. While this mutation is typically associated with favorable outcomes, poor outcomes raise the question of extra-cochlear, central deficits. This work aims to identify neural oscillatory features of language development variabilities as well as etiology-specific neural features with the GJB2 mutation in early-implanted children.

Methods: We will recruit approximately 100 children (ages 4–17) with congenital bilateral hearing loss and early CIs (first CI before 30 months). EEG (electroencephalogram) will be recorded while children watch cartoon videos (12 minutes in total). We will compute theta-gamma phase-amplitude coupling (PAC) as an index of speech-related neural activity. Retrospective clinical data from the speech therapist's report will be used to compute language composite scores using Confirmatory Factor Analysis (CFA), starting from the two-year post-implant follow-up. A linear mixed-effects model (LMM) will assess the relationship between etiology, neural oscillatory features, and language outcomes. A mediation analysis will evaluate whether PAC mediates the effect of etiology (GJB2 vs. non-GJB2) on language development.

Results: Among 141 children currently identified, 62 have GJB2 mutations, confirming it as the most common genetic etiology. Preliminary analysis of speech-language follow-up data (CAP, SIR, and expressive communication scores) reveals substantial interindividual variability in outcomes. After controlling for age of implantation, the GJB2 group performs better than other etiologies across all three assessments. However, of the children falling in the lowest 15% of language performance, six carry GJB2 mutations, indicating notable within-group variability. We expect upcoming EEG data to reveal that theta-gamma PAC during speech perception predicts speech outcome and mediates the relationship between etiology and language ability.

As EEG data collection is ongoing, neural findings will be presented at the conference.

Conclusions: This study will be among the first to link neural oscillatory mechanisms to language variability in children with early CIs. By combining clinical, neural, and genetic data, we aim to uncover etiology-specific neural pathways that contribute to speech and language outcomes. Clinically, the findings can help to identify children at risk of poor outcomes and guide future interventions, including gene therapies, based on neurophysiological and genetic profiles.

T160. A Consideration of How to Control the Influence of Non-Auditory Nuisance Factors on Digit-Perception Tasks

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Background: A common tool for probing auditory function and perception is to ask listeners to identify spoken digits in the presence of masking or distracting sounds. Examples include the use of digit triplets in speech-shaped noise to screen for hearing loss, or of spatially separated streams of digits to reveal temporal processing deficits which may be associated with cochlear synaptopathy. Yet speech-perception tasks are affected by factors beyond auditory function, such as cognition, motivation, and language. The present work aims to assess the extent to which digit-perception tasks are influenced by a range of potential nuisance factors, how these influences vary with masker characteristics, and how best to account for the variability in thresholds caused by factors not directly related to the research question under investigation.

Methods: The dataset is composed from 220 otologically healthy teenagers, gathered at baseline of a longitudinal study. The influences of non-auditory nuisance factors should emerge more clearly in this homogeneous cohort than in older and more diverse populations. Participants completed three digit-perception tasks, each with a diotically presented digit-triplet target. The maskers were: (A) diotic speech-shaped noise, (B) spatially separated 10-talker babble, and (C) two spatially separated digit triplets, distinguishable from the target only via spatial cues. Condition A represents traditional digits-in-noise testing; condition B greater ecological validity; and condition C maximum reliance on spatial processing. Performance was tested for relations to educational attainment, auditory working memory, musicianship, and socio-economic status.

Results: Perception of digits in conditions A and B was generally unrelated to the tested nuisance factors. In contrast, the task relying solely on spatial cues (C) showed significant associations with educational attainment, musicianship, and socio-economic status in univariate analyses. Multiple linear regression models revealed that educational attainment had the strongest influence on performance, with a smaller contribution from musicianship. Model comparisons indicate that after controlling for confounding factors, an apparent association between musicianship and listening performance was reduced by 40% and was rendered non-significant.

Conclusions: Digits-in-noise and digits-in-babble tasks appear relatively free of influence from the tested nuisance factors, even when some spatial cues are present; not so for a digit-discrimination task reliant solely on spatial cues. Observed relations between musicianship and

non-musical auditory performance are sometimes attributed to “training effects” from musical practice, but may also reflect confounding factors; teenagers in our cohort who play music are more privileged and highly educated than those who do not. It may be that a relatively blunt measure, such as educational attainment, captures a more complex interaction of socio-economic status, education and motivation to perform well in a laboratory task. It may be advantageous to include such a variable in future analyses of speech perception, as a proxy for unwanted variance in the data.

T161. White Matter Density Within the Central Auditory Pathways Predicts Degraded Speech Perception Abilities by Constraining “Top-Down” Auditory Function

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Background: Difficulty understanding speech-in-noise (SIN) is a common problem even in young, normal-hearing listeners suggesting brain mechanisms beyond the cochlea play a critical role in cocktail party speech perception. Yet, a detailed understanding of the central auditory system and its relation to hearing behaviors is virtually unknown. Using a multimodal, neuroimaging approach combining EEG, MRI tractography, and behavior, we assessed how functional and structural properties of the brainstem-cortical auditory pathways map to individual differences in SIN understanding.

Methods: We recorded source-level, anatomically constrained frequency-following responses (FFR) and event-related potentials (ERPs) to speech via high-density EEG while listeners performed rapid SIN perception tasks. Source and functional connectivity analysis parsed speech-evoked responses into brainstem (BS) and auditory cortical (AC) sources allowing us to examine the neural encoding and transmission of speech along the brainstem-cortical neuroaxis. Microstructural properties of the circuit were assessed via diffusion weighted imaging (DWI) of prominent white matter fiber bundles.

Results: Noise hindered speech perception and neural encoding at both brainstem (FFR) and especially cortical (ERPs) levels. Directed functional connectivity computed from EEGs showed stronger efferent/feedback (AC→BS) than afferent/feedforward (BS→AC) neural transmission that was left lateralized, suggesting heavier engagement of the corticofugal efferent system originating in the language hemisphere. Electrophysiological findings were paralleled in anatomical measures. Larger DWI axonal density was associated with better SIN performance overall, with denser fibers in left hemisphere and correlations with efferent but not afferent EEG functional connectivity.

Conclusions: Our neuroimaging findings expose new structure-function-behavior interactions in the lemniscal hearing pathways that predicts complex speech perception skills. Noise reduces neural representation of speech at multiple stages of auditory processing but these declines are partially counteracted by corticofugal feedback via the descending auditory system. Critically, central auditory efferent function appears dependent on the underlying brain anatomy. This may constrain auditory perceptual skills and plasticity that draw upon top-down processes (e.g., learning), thereby accounting for a range of perceptual skills even in young, normal-hearing individuals.

T162. Time Constant of Auditory Adaptation to Noise: Psychoacoustic and Neurophysiological Evidence With Clicks

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Background: 'Adaptation to noise' refers to the better detection or recognition of a signal when it is delayed in the noise. This probably reflects the auditory system's ability to adjust its settings to optimise the neural encoding of information in acoustically challenging venues such as noisy cafeterias and restaurants. While psychoacoustic and computational studies have examined this effect, the underlying physiological mechanisms remain unclear. This study aimed to estimate the activation time constant of these mechanisms and, for the first time in humans, to test whether the neurophysiological representation of a target sound improves when its onset is delayed relative to noise using non-invasive methods.

Methods: Speech intelligibility thresholds (50% recognition) were measured in 15 normal-hearing adults (7 females; 19–46 years) for vocoded words presented at 50 ms (early), 800 ms (middle), and 1600 ms (late) after the onset of speech-shaped noise presented at 60 dB nHL (63.7 dBA). Auditory brainstem responses (ABRs) were recorded in 12 normal-hearing adults (7 females; 20–40 years) using bursts of clicks presented 50 ms (early) and 800 ms (delayed) after noise onset. In addition, click detection thresholds in noise under the same conditions are currently being measured in a further cohort of normal-hearing adults (> 10 participants expected by February 2026).

Results: Speech intelligibility thresholds improved by 2.4 dB ($p < 0.001$) and 2.9 ($p < 0.001$) in the middle and late conditions, respectively, compared to the early condition. The two delayed conditions yielded statistically comparable benefits ($p > 0.05$). ABR morphologies were statistically indistinguishable between early and delayed conditions, with no significant differences in wave I or wave V amplitude or latency ($p > 0.05$ in all analyses).

Conclusions: Consistent with previous literature, the improvement in speech intelligibility with delayed target presentation confirms a robust adaptation-to-noise effect. The lack of additional benefit at 1600 ms re 800 ms suggests an activation time constant below 800 ms, consistent with mechanisms such as medial olivocochlear reflex activation and dynamic range adaptation of neurons along the auditory pathway. The absence of an ABR effect replicates previous animal findings and indicates that clicks may not be optimal for objectively characterising these processes. Ongoing psychoacoustic experiments will clarify whether click adaptation to noise occurs at the behavioural level. Stimuli based on synthesized speech may ultimately provide greater sensitivity for probing the neurophysiological basis of this phenomenon.

T163. Role of Listening Effort and Linguistic Experience in Predicting Hearing Difficulty in Listeners With Typical Hearing

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Background: Hearing difficulty (HD) in individuals with typical hearing (TH) is reported to affect 10-15% of the population and is characterized by poor self-reported or behavioral hearing performance. There are many suggested underlying causes, possibly due to the multifaceted nature of hearing and individual diverse listening experiences. The aim was to identify behavioral indicators of HD by investigating how it relates to specific auditory sub-domains, listening effort (LE), and prior auditory experiences such as second-language acquisition and music training.

Methods: Participants (N=91) were recruited from a university participant pool, as part of a larger online study regarding personality and hearing measures (N=321). They were categorized into an HD group (N=31; 28F; mean age=19.9) and a control group (N=60; 33F; mean age=20.1) based on pre-screening scores. Group assignment was determined using the 15-item Speech, Spatial, and Qualities of Hearing Scale (15iSSQ) and a Digit-in-Noise (DIN) test at -12 dB signal-to-noise ratio: the HD group was defined by 15iSSQ scores < 7.9 (M=6.99) and DIN accuracy $\leq 60\%$ (M=40%), while the control group had 15iSSQ scores ≥ 9.0 (M=9.46) and DIN accuracy $\geq 80\%$ (M=91.67%). All participants subsequently completed the Hyperacusis Questionnaire (HQ), the NASA-Task Load Index (NASA-TLX) to measure listening effort (LE), and a background survey on linguistic experience and music training.

Results: Significant differences were found between the two groups across multiple domains. The HD group reported poorer performance on all 15iSSQ sub-domains (Speech, Spatial, Qualities; all $p < .001$) and HQ sub-domains (Attention, Social, Emotional; all $p < 0.03$) than the control group. The HD group also reported significantly higher NASA-TLX scores than controls in the sub-domains of temporal demand ($p = .003$), effort ($p < .001$), and frustration ($p = .001$). These results suggest HD listeners experience challenges beyond speech performance, such as hearing quality, attention, emotion, and social life.

A logistic regression model significantly predicted HD status ($p < .001$). Gender (Odds Ratio [OR] = 8.44, $p = .006$), the number of languages acquired (OR = 5.06, $p = .006$), and the self-reported LE (NASA-TLX) score (OR = 1.05, $p = .001$) were significant predictors of having HD, accounting for 29.49% of the total variance. In summary, HD as measured by 15iSSQ and DIN is more likely to occur among females, multilingual individuals, and those reporting higher LE in noise. In contrast, age, race, and years of music training were not significant predictors.

Conclusions: Take-away message: HD affects not only speech perception but also the overall quality of life for young adults with TH. Key factors increasing HD odds include female sex, multilingualism, and elevated LE, which can be used as auxiliary diagnostic indicators for clinicians. Further investigation is needed to develop effective interventions for this understudied issue.

T164. Role of Implicit Auditory Memory During Speech in Noise Listening

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Background: Speech perception in noise (SPIN) remains a major challenge for people with hearing loss, even with amplification. Rehabilitation methods such as auditory training are recommended to ameliorate these challenges, but evidence supporting these interventions is

mixed. Training the associated cognitive/perceptual systems that support real-world listening by decreasing cognitive effort and fatigue have been proposed as alternative approaches. Specifically, the role of statistical learning mechanisms on perceptual systems is particularly promising, due to their implicit nature and role in shaping neural representations of speech. Recent work studying the implicit auditory memory of tone sequences has revealed long-lasting behavioral consequences, highlighting its potential in auditory training. Here, we test whether implicit memory would also provide benefit to SPIN.

Methods: Fifty-seven right-handed, normal hearing, native English-speaking participants were recruited online using Prolific and tested using a custom-built open-source psychophysical testing platform. Testing occurred over two sessions separated by one week. Experimental tasks included a replication of a previous study (Bianco et al., 2020) using pattern recognition of tone pip sequences to confirm the influence of implicit memory over a 1-week period, and a sentences in noise task to determine if benefit extended to speech stimuli. In both, reaction time was measured as a proxy for listening effort and select stimuli units were repeated across the tasks to leverage implicit memory.

Results: Reaction time for repeated tone pip sequences decreased between the first and last block of session one compared to non-repeated stimuli conditions, suggesting a benefit due to implicit auditory memory. This benefit was maintained 1-week later at session two, indicating that increased exposure to the stimuli allowed the perceptual system to create partial memories that benefited pattern recognition in the short term. Reaction times for repeated conditions in the sentences in noise task were only decreased at higher (easier) signal to noise ratios, suggesting initial exposure to stimuli must be unobscured for implicit memory to support listening abilities.

Conclusions: Our results replicate an earlier study showing the benefits of implicit memory in statistical learning over a 1-week period. In addition, we extend these results to show that implicit auditory memory can benefit speech listening, but only under certain conditions. The implicit aspect of this cognitive mechanism is especially appealing for clinical application into aural rehabilitation methods, as current interventions receive very low patient compliance. Targeting a passive phenomenon within auditory training can make rehabilitation more appealing and accessible to patients who struggle to hear in noise, even with amplification. Taken together these results can inform parameters for auditory training programs and encourage the consideration of associated cognitive systems into training as well.

T165. Statistical Prediction Influences Neural Latencies and Schema-Based Speech Separation From Noise

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Background: Listening in noisy environments depends on the brain's ability to segregate an auditory scene into distinct perceptual streams. Stream segregation is aided by statistical regularities at various levels. Acoustic regularities like comodulation and harmonicity, present in natural sounds like speech, provide strong cues for bottom-up perceptual grouping. Higher-level regularities like linguistic structure must be learned to form a mental "schema" (stored knowledge) of statistical patterns in the acoustic environment. Schema-based grouping may rely

on predictive modeling of the input sound, but a direct mechanistic link between statistical prediction and segregation has not been established. One view in the field is that schemas aid primitive perceptual organization by supporting sequential grouping of elements of a learned sound pattern across time. A competing view is that schemas stabilize already formed sound streams, aiding selective attention to a target stream via template matching. Although the term “schema-based segregation” encapsulates these two distinct accounts, the degree to which these different perceptual mechanisms shape schema-governed listening is unknown. Furthermore, the neural processes underlying schema-based segregation are unclear.

Methods: We combined electroencephalography (EEG) with behavior in normal-hearing listeners (N=50) to gain insight into the mechanisms by which statistical regularity aids segregation. Following established statistical learning paradigms, we exposed listeners to sequences of speech syllables with specific syllable-transition probabilities. Subjects were asked to detect and silently count the number of times they heard a particular target syllable. As subjects learn the statistical structure of the syllable sequences they hear, they should become better at detecting a particular target syllable because they will be able to predict it from the preceding syllables. Post exposure, subjects were tasked with attending to one of two simultaneous speech streams and counting target syllables presented in the attended stream; by manipulating the syllable-transition probabilities in the attended and competing speech streams, we explored how predictability of each stream influences segregation.

Results: Target-syllable detection is better when between-syllable transition probabilities in the attended stream predict the target. This prediction benefit for speech perception is greater in competition than in quiet, suggesting that statistical prediction aids schema-based segregation. We found no significant effect of masker predictability on performance, suggesting that, at least for predictable syllable sequences, schemas serve primarily to stabilize existing perceptual streams. The parietal P300 EEG event-related potential accompanying target-syllable detection occurs earlier when the target is predictable, and, overall, neural encoding of the attended speech (indexed by intertrial coherence) is stronger in the predictable condition. Neural correlates of predictive processing are also evident in the absence of target detection.

Conclusions: Our findings suggest that statistical prediction influences schema-based speech separation from noise. Neural correlates of predictive processing are clear both in quiet and in acoustic competition.

T166. Clarifying the Role of Temporal-Coherence Processing in Speech Intelligibility in Noise

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Background: A rich literature on grouping, comodulation masking release (CMR), and cross-channel modulation interference supports the theory that temporally coherent sound modulations help bind together sound elements across distinct frequency channels to form a perceptual object. This in turn can help us perceptually segregate (separate) different sound sources in an auditory scene, like speech from noise. A consequence of this theory is that masker components that are temporally coherent with the target but in distinct frequency channels not driven by the target may interfere with target perception. Recent work that manipulated between-frequency temporal

asynchrony (independently for the target speech and masker) raises the possibility that temporal coherence may not be as critical a cue for segregating speech as it is for the non-speech stimuli used in classic CMR studies (Lee and Oxenham, 2024). In the current study, we tested this possibility by directly manipulating the temporal coherence between masking noise and target speech across channels, and between different frequency components of the noise masker. In line with the temporal-coherence theory and classic tone-based CMR results, we hypothesized that speech intelligibility is (i) poorer when the masker (M) is temporally coherent with the target (T) speech (TM-COH condition) compared to when they are incoherent with one another (TM-INCOH), and (ii) poorer when the different frequency components of M are temporally incoherent with each other (achieved by phase shifting M's envelope across frequency bands) even when they are incoherent with T (TM-INCOH-MPS), compared to the TM-INCOH condition.

Methods: We used in-lab (N=19) and online (N=30) psychophysics to test our hypotheses. Subjects listened to running speech (QuickSiN and Matrix sentences) in speech-shaped noise presented at different signal-to-noise ratios (SNRs) in TM-COH, TM-INCOH, and TM-INCOH-MPS conditions. In all cases, the speech and noise were vocoded such that they occupied different frequency bands (frequency separation twice the conventional critical bandwidth estimates) to minimize energetic masking, allowing us to isolate the role of across-channel temporal-coherence processing. We derived psychometric curves for percent words correct as a function of SNR, and computed the SNR threshold difference at a fixed percent correct between TM-COH and TM-INCOH and between TM-INCOH-MPS and TM-INCOH as our speech-based measures of temporal-coherence processing.

Results: Preliminary data show that speech intelligibility is significantly worse in TM-COH compared to TM-INCOH, suggesting that across-channel temporal coherence between target speech and background noise significantly degrades performance. The performance difference between TM-INCOH-MPS and TM-INCOH is smaller, suggesting that across-channel temporal asynchrony between different frequency components of the masker (from phase shifting the envelopes) has a small effect on speech segregation.

Conclusions: Our data support the interpretation that temporal coherence is a strong cue for segregating speech from background noise.

T167. Learning on Non-Native Phonetic Classification is Differentially Sensitive to Visual Cognitive Demands During Different Learning Stages

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Background: Perceptual abilities improve with training, but that learning can be disrupted when two different perceptual tasks are trained within the same session. Here we asked whether two previously observed patterns of learning disruption also occur when one of the perceptual tasks is replaced with an unrelated cognitive task. In one pattern, learning on one perceptual task (task A) is disrupted by subsequent training on another perceptual task (task B). Such retrograde learning interference suggests that training on task B usurps resources that are necessary for consolidating the learning on task A. If retrograde interference also arises when task B is a cognitive task, it would suggest that the resources necessary for consolidation can be usurped by

cognitive, and not just perceptual, demands. In another pattern, learning on task A is disrupted when all trials on task A are completed before the trials on task B begin (blocked training) but not when the trials of task A and task B alternate every few minutes (interleaved training), or vice versa (learning during blocked but not interleaved training). Such blocked-versus-interleaved training differences suggest that learning on task A passes through separate stages that require different resources to complete. If blocked-versus-interleaved training also yields different outcomes when task B is a cognitive task, it would suggest that the resources necessary for completing different learning stages can be usurped by cognitive demands in one learning stage but not another.

Methods: Five groups of adult native-English speakers (n=8-10/group) practiced a target non-native phonetic classification task with feedback for 120 trials either only (control group) or in combination with separate period(s) of performance of an attention-demanding visual task: Rapid Serial Visual Presentation (RSVP). Different groups completed the RSVP task in silence, or with the phonetic stimuli playing in the background. The phonetic and RSVP tasks were either presented consecutively, with the phonetic task first (blocked regimens), or alternated every ~6 minutes (interleaved regimens). We assessed each group's performance immediately before and after a single training session.

Results: Practicing the phonetic task alone yielded learning. That learning was clearly disrupted by subsequent performance of the RSVP-in-silence task, but not by the RSVP-with-sound task. In contrast, interleaving the phonetic and RSVP-in-silence tasks yielded learning, though not significantly more learning than blocked training, while interleaving the phonetic and RSVP-with-sound tasks clearly disrupted learning.

Conclusions: (1) Resources necessary for consolidation can be usurped by cognitive, and not just perceptual, demands. (2) The loss of resources for consolidation due to cognitive demands can be mitigated by additional task-relevant stimulus exposure. (3) Resource requirements, their susceptibility to cognitive demands, and mitigating factors differ across different learning stages.

T168. Learning on Musical-Interval Discrimination Recovers From Retrograde Interference at Different Rates for Different Interval Comparisons

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Background: Learning on one perceptual task can be disrupted by subsequent training on another. Such retrograde learning interference suggests that training on the second task usurps resources that are necessary for stabilizing, or consolidating, learning on the first task. However, any given second task generates retrograde interference on only a select set of first tasks, suggesting that the resources required for consolidation are shared among limited sets of tasks and hence that consolidation is a task-specific process. If so, there should be cases in which the same second task has different influences on different first tasks even when the first tasks are intermixed during training. Here we report data consistent with this prediction from an investigation of learning on musical-interval discrimination.

Methods: We trained normal-hearing young adults on musical-interval discrimination. On each two-observation forced-choice trial, listeners chose between random examples of a target musical interval and one of three intermixed foil intervals. They thus encountered three different trial types or “tasks”. The target interval was either a 4th (foils: 3rd, 5th, 6th) or a 7th (foils: 5th, 6th, octave). Three different groups (n=7-8/group) completed three daily training sessions on the 4th only, on the 4th followed 0h later by the 7th, or on the 4th followed 6h later by the 7th.

Results: The 4th-only group improved statistically significantly on all three trial types: the 4th vs. the 3rd, 5th, and 6th. In contrast, the 4th-0h-7th group did not improve significantly on the 4th for any trial type and improved significantly less on every trial type than the 4th-only group, revealing retrograde learning interference. Crucially, the 4th-6h-7th group did not improve significantly on the 4th vs. the 3rd or 5th, but did improve significantly on the 4th vs. the 6th. They also improved significantly < the 4th-only group on the 4th vs. the 3rd, but not on the 4th vs. the 5th or 6th. Thus, the recovery rate from retrograde learning interference was slowest for the 4th vs. the 3rd, intermediate for the 4th vs. the 5th, and fastest for the 4th vs. the 6th.

Conclusions: Learning from training on musical-interval discrimination with one target interval (a 4th) was disrupted by subsequent training with a different target interval (a 7th). This retrograde learning interference decreased at different rates for three different intermixed trial types (“tasks”) as the time between the training on the two target intervals increased. The result aligns with previous evidence of task-specific retrograde interference in perceptual learning, but adds that this task specificity remains when different first “tasks” are intermixed during training. Thus, it appears that, during perceptual training, trials from intermixed tasks are sorted by task and consolidate separately, thereby forming task-specific memories.

T169. Is the Stream Nearby Active? Listening to the Hydrodynamics of a River in a Natural Environment

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Background: Streams are dynamic systems whose acoustic signature varies with discharge, velocity, and geo-morphological factors. The present study aims to investigate the extent to which and how human listeners discriminate hydrodynamic changes in a river in a natural environment.

Methods: To address this issue, sound recordings were conducted for a stream located in a Corsica over a 400m segment using eight microphones located on the riverbed and spaced apart along this segment every 50m approximately. Recording site is part of a Mediterranean-mountain biome marked by dense maquis shrubland, holm oak and chestnut forests and rugged valleys shaped by steep relief, where the recorded stream creates diverse riparian habitats hosting rich bird, insect, and amphibian communities. The study site was located downstream from a hydroelectric powerplant where controlled releases of water produced rapid and marked changes in discharge rates over a 1h period. Estimated discharge data were collected in parallel to the acoustic recordings at the powerplant site, allowing precise alignment of physical and auditory measures. This database provides a range of ecologically-valid acoustic stimuli reflecting variations in flow discharge, current speed, and local facies (e.g., riffles vs. pools) and influence

of local vegetation and fauna. Stimuli are then presented online under headphones to normal-hearing participants asked to perform psychophysical tasks aiming to assess auditory discrimination performance of hydrodynamic changes in the stream state. Here, an oddity (forced-choice) paradigm is used where participants are asked to discriminate hydrodynamic changes across intervals. All stimuli are normalized in root-mean-square power to force listeners to rely on the informational content of the stimuli only, knowing that level cues are strongly influenced by distance to the stream.

Results: Psychophysical data will be discussed in light of modelling data obtained by simulating an auditory model extracting texture statistics at the output of cochlear and modulation filters in response to the same database.

Conclusions: Previous work has shown that temporal-envelope statistics guide the detection of water in natural soundscapes. This study extends that framework to the discrimination of hydrodynamic changes in a river setting. Using ecological recordings and online psychophysics, we will test whether similar auditory cues underlie sensitivity to variations in discharge, velocity, and facies. Conclusions will be interpreted at the light of the previous study, with the psychophysical data.

T170. Is There a Stream Nearby? Auditory Detection of Water in a Natural Environment

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Background: Water is an essential nutrient for living organisms. Surprisingly, knowledge is lacking about the way human beings process acoustic information conveyed by water in natural settings with their ears and their auditory brain. Our research aims to better understand how humans perceive liquid sounds within natural soundscapes, hypothesizing that water holds a particular perceptual role for human observers due to its ecological importance. To achieve this, we sought to address various objectives: (i) understanding which auditory cues and mechanisms humans use for the detection of water sounds in natural environments, (ii) determining if these cues vary as a function of distance, moments of the day and seasons.

Methods: To address this issue, a Mediterranean stream was recorded at varying distances from a stream segment (hereafter called ‘the source’), times of day (morning, noon, evening), and seasons (spring, summer) in a nature reserve (a forest). Human participants tested online under headphones performed a two-interval forced-choice task in which they had to determine presence of water. On a typical trial, target and standard stimuli corresponded to acoustic samples recorded over a range of 0-100m distances to the source on a given transect, and samples recorded at 150 or 200m from the source on a different transect, respectively. To eliminate memory effects, a single-trial procedure was used where each participant was tested on a single trial only.

Results: The results confirmed that auditory detection of water depends strongly on distance to the source, consistent with expectations from spherical spreading and masking effects produced by the ambient-noise field. Seasonal changes associated with variations in sound-propagation

conditions and masking effects produced by geophysical events and animal vocalizations also influenced water detection, whereas diel variations had no significant effect on auditory judgments. The empirical psychometric functions were compared to simulated psychometric functions obtained by running the same task with the same stimuli with a computational model of the auditory system. This model implemented a cochlear filterbank followed by a modulation filterbank and a decision stage based on a template-matching strategy. The best fit to the human data was obtained when the auditory model based its decisions upon skewness and kurtosis of envelope fluctuations at the output of cochlear filters, and cross-channel correlation patterns at the output of modulation filters.

Conclusions: These results suggest that human listeners detect presence of water in natural settings by analysing the sparsity and coordination of amplitude-modulation cues conveyed by natural soundscapes, consistent with previous work demonstrating a role of these texture statistics in water identification (McDermott and Simoncelli, 2011).

T171. Hearing-Impaired Deep Neural Networks Predict Real-World Hearing Difficulties

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Background: Computational models that perform real-world hearing tasks using cochlear input could help link the peripheral effects of hearing loss to real-world perceptual consequences. Deep artificial neural networks trained to perform ecological hearing tasks using simulated cochlear input reproduce many aspects of normal hearing, but it is not clear whether such models can also account for impaired hearing. Here, we investigated whether simulating the peripheral effects of hearing loss in a model optimized for everyday tasks can predict the perceptual behavior of hearing-impaired listeners.

Methods: To model normal hearing, we trained a single deep neural network model to localize and recognize speech, voices, and environmental sounds using simulated auditory nerve representations of naturalistic scenes. Once trained, we simulated hearing loss in the model's peripheral input and asked if it could predict the speech intelligibility and psychoacoustic thresholds of hearing-impaired listeners. We used the Clarity Challenge dataset (a collection of 24120 speech-in-noise scenes and intelligibility scores from 33 hearing-impaired listeners) to test whether the model could account for differences across individual listeners. To disentangle the perceptual consequences of distinct types of hearing loss, we also separately measured the effects of inner hair cell (IHC), outer hair cell (OHC), and auditory nerve fiber (ANF) loss on our model's behavior.

Results: When equipped with simulated healthy cochleae, the model accounted for several aspects of binaural speech perception in humans with normal hearing, reproducing the effects of noise, reverberation, and spatial separation between speech and noise. When input was instead provided by simulated damaged cochleae, the model's performance resembled that of humans with hearing loss: speech recognition deteriorated (especially at low SNRs) and spatial release from masking was reduced. Individualizing the hearing loss simulation to match listeners' unique audiograms enabled the model to account for ~50% of the explainable variance in speech intelligibility scores across listeners in the Clarity Challenge dataset, improving upon the 30%

explained by the Hearing-Aid Speech Perception Index (HASPI; a standard speech intelligibility metric that does not perform auditory tasks). Simulations of plausible and idealized hearing loss phenotypes (i.e., combined OHC and ANF loss vs. isolated OHC or ANF loss) suggest that both OHC and ANF loss contribute to real-world hearing difficulties, but they produce distinct behavioral outcomes in psychoacoustic listening tests.

Conclusions: Machine-learning-based models that generate behavior from simulated auditory nerve input can predict aspects of hearing-impaired behavior and may help disentangle the perceptual consequences of different types of hearing loss.

T172. Quality Estimation of Human Frequency-Following Responses Using the Fsp Algorithm

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Background: The frequency-following response (FFR) is a valuable tool for studying auditory processing, but its reliability is limited by variability in background neural activity and individual differences in recording conditions. Traditional averaging of a fixed number of sweeps does not guarantee consistent response quality. A similar challenge in auditory brainstem response (ABR) recordings led to the development of the Fsp (F statistics over a single point) algorithm, which objectively quantifies response quality using variance ratios. Given the shared characteristics of ABR and FFR, the purpose of this study was to apply the Fsp algorithm to FFR with the intent of providing an automated, observer-independent method for improving detection accuracy and optimizing data collection in both research and clinical settings.

Methods: Fifteen normal-hearing college students participated in ABR and FFR recordings in separate, randomized sessions within a sound booth. ABRs were elicited by acoustic clicks at 0, 20, 40, and 60 dB nHL, and FFRs by a 150-ms English vowel /i/ with rising intonation at 60 dB nHL. Continuous EEG was recorded with scalp electrodes, amplified, filtered, segmented into sweeps, and averaged. Fsp values were calculated as the ratio of the variance in the averaged time waveform to the variance of the EEG values at a single point (SP) across all recording sweeps. Three-way ANOVAs assessed the effects of stimulus type, sweep count, and SP location.

Results: In ABR recordings, Fsp values increased more rapidly at higher stimulus intensities, exceeding the 3.1 detection criterion with fewer sweeps (about 1,250 sweeps at 60 dB nHL; about 1,500 sweeps at 40 dB nHL), whereas low intensities required more sweeps and control conditions showed no increase. For FFR, experimental conditions surpassed the criterion at about 1,750 sweeps, whereas control conditions remained flat. Three-way ANOVAs revealed significant effects of intensity, experimental/control condition, and sweep count for both ABR and FFR measures, but no significant effect of SP location.

Conclusions: The Fsp algorithm is a reliable and effective tool for objectively assessing both ABR and FFR. By quantifying response quality through variance analysis, it enables automated, observer-independent detection of neural activity, thereby enhancing both clinical diagnostics and auditory neuroscience research. Clinically, the Fsp algorithm could streamline neonatal and pediatric hearing screenings, reduce inter-observer variability, and improve diagnostic accuracy. In research, it offers a consistent, quantitative measure for evaluating the reliability of auditory

evoked potentials, supporting studies on auditory processing disorders, speech perception, and aging.

T173. Detection Thresholds of Multiple Gaps in Difficult-To-Detect Conditions

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Background: Gap detection threshold (GDT) is an index for assessing auditory temporal resolution. An objective GDT measurement method was developed using auditory steady-state response (ASSR) elicited by silent intervals at 40 Hz or 80 Hz within broadband noise, termed “GapASSR” [Kadowaki et al., 2022; Kadowaki et al., 2023]. The noise stimulus includes silent intervals of 25 or 12.5 ms, corresponding to 20 or 40 gaps in a 500-ms stimulus. Our previous study with normal-hearing listeners demonstrated that detection thresholds decrease with the number of gaps or shorter gap onset asynchrony (GOA). Thus, GDT estimated by GapASSR is expected to be lower than that from traditional stimuli (e.g., 500 ms of noise with one gap). However, it is unclear if this trend persists where gap detection is challenging. This study aims to determine whether similar trends occur under such conditions.

Methods: To simulate conditions where gaps are difficult to detect, an experiment was conducted using stimulus sounds with reduced amplitude instead of silence. The carrier was a low-noise noise [Kohlrausch et al., 1997] at a sound pressure level of 65 dB. The experiment featured three gap conditions: silent, -6 dB attenuation, and -2.5 dB attenuation. In Experiment 1, a constant GOA of 25 ms was used with varying numbers of gaps set at 1, 4, and 8. In Experiment 2, the gaps were fixed at eight while the GOAs varied at 12.5 and 100 ms. Stimulus lengths were 500 ms for Experiment 1 and 1000 ms for Experiment 2. A three-interval, three-alternative, forced-choice, 2-up and 1-down procedure was employed for measurements. Thresholds were measured once for each condition, with participants having normal hearing.

Results: Two-way analyses of variance were conducted for Experiment 1, using gap condition and number of gaps as factors, and for Experiment 2, using gap condition and GOA as factors. Both analyses showed significant main effects, but no interactions were found.

Conclusions: The analyses of variance revealed trends consistent with our previous studies, even under difficult-to-detect conditions. Future work will evaluate GapASSRs in these settings.

T174. Using Eye Gaze to Explore Decision-Making During Binaural Processing: Comparing Temporal Fine Structure and Envelope Cues

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Background: Spatial hearing skills are supported by binaural cues, including interaural differences in timing and level (ITD and ILD, respectively). Just-noticeable difference (JND)

thresholds are commonly used to estimate performance, but JNDs alone fail to capture underlying decision-making processes. Eye gaze paradigms provide a unique opportunity to access the time course of decisions, and the strategies listeners adopt during the processing of binaural cues. Our work is designed to compare binaural processing in individuals with typical hearing (TH) and with bilateral cochlear implants (CIs); here, we present data from TH individuals, using stimuli designed to simulate CI stimulation, with special consideration of temporal constraints in single-electrode stimulation, where access to temporal fine structure (TFS) and envelope (ENV) cues differs depending on apical versus basal electrode locations.

Methods: Participants with TH (ages 18-25 years) were tested to extract eye gaze and JNDs. ITD or ILD varied in magnitude across the physiologically relevant range. TFS stimuli (100/s), and ENV stimuli (4000/s, modulated at 125 Hz), had a notched noise to limit off-frequency listening, with notches centered at 500 Hz and 4000 Hz, respectively. Behavioral JNDs were extracted from participants' reporting of sounds perceived to the right vs. left. Eye gaze data were analyzed as trajectories of percent looks to the target side (latency, slope, and asymptote), quantifying the time course of decision-making. Fixation switches between target and non-target sides were calculated to quantify uncertainty in decision-making before the final response was made.

Results: JNDs were lower for ITD-TFS than ITD-ENV, while ILD-TFS and ILD-ENV produced similar JNDs. Logistic fits to eye gaze trajectories showed that slopes increased with cue magnitude for all conditions except ITD-ENV, where slopes did not vary systematically with cue magnitude. For ILD- TFS and ILD-ENV, the slope was similar across cue types, indicating comparable decision-making processes. Latency decreased systematically with cue strength in all conditions except ITD-ENV, where latencies remained longer even at larger cue levels.

Conclusions: Processing demands differ for TFS and ENV cues in the ITD domain, but not for ILDs. TFS-ITDs had lower JNDs, steeper slopes, and shorter latencies compared with ENV-ITDs. ILD cues were processed more similarly across TFS and ENV, indicating that level differences remain robust even under CI-like stimulus constraints. Results show that these CI-inspired stimuli elicit cue-specific decision strategies and provide gaze-based markers of uncertainty beyond JNDs, establishing a translational baseline for comparison with CI users.

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T175. Tests of Auditory Temporal Resolution: Use of Bimodal Distributions for a Bayesian Adaptive Procedure

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Background: Auditory temporal resolution plays a critical role in the everyday experience of listening to complex acoustic patterns. The two most commonly used methods for assessing auditory temporal resolution are gap detection and amplitude modulation (AM) detection. In an

attempt to develop a standardized clinical test of auditory temporal resolution, we used Zippy Estimation by Sequential Testing (ZEST), a Bayesian threshold estimation procedure, to measure gap and AM detection thresholds. In our recent studies (Mori, 2023, 2024, submitted), we conducted computer simulations and human experiments to show the efficiency and precision of ZEST for measuring gap and AM detection thresholds. To further improve the performance of ZEST, we used bimodal distributions, which combined the probability density functions (pdfs) of normal-hearing (NH) and hearing-impaired (HI) participants, as the initial pdfs. In the present study, we conducted computer simulations to examine the efficiency and the precision of the above method.

Methods: The present study consisted of two stages. In the first stage, we estimated the pdfs of gap and AM detection thresholds separately for 121 NH participants and 23 HI participants using data obtained in our recent study (Mori et al., submitted). We applied kernel density estimation to this data to estimate the pdfs of the thresholds for the NH and HI participants. We fitted Gaussian distributions to these pdfs using the least-square method. In the second stage, we conducted computer simulations of ZEST with bimodal distributions as the initial pdfs, which combined the best-fit Gaussian distributions for the NH and HI participants. In the simulations, we manipulated the NH to HI distribution ratio, to identify the optimal ratio in terms of ZEST performance.

Results: Gaussian distributions fitted the gap and AM detection pdfs of the NH and HI participants quite well. The simulations in which the bimodal distributions were used as the initial pdfs showed that when the ratio favored the NH (or HI) pdfs, it improved the efficiency and precision of threshold estimation for the NH (or HI) participants; i.e., within 15 trials the threshold estimates reached the ‘true’ threshold values assumed in the simulation.

Conclusions: Computer simulations demonstrated that the use of bimodal distributions as initial pdfs improved the performance of ZEST for gap and AM detection threshold measurement. In particular, using different ratios of the pdfs for the NH and HI listeners provided flexibility for ZEST measurement, which would be useful in the clinical setting. We are now conducting human experiments using the same methods as were employed in the simulations.

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T176. Perceptual Expertise: Amplitude Modulation Rate Discrimination at Detection Threshold

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Background: Many simple perceptual skills improve with a few days of training. However, weeks or months of practice may be required to reach a level of expertise on complex tasks (Watson, 1980). Here, we explored how gerbils attain expertise on a difficult task: amplitude modulation (AM) rate discrimination at extremely small AM depths, similar to the smallest depths used during vocal communication.

Methods: We used an appetitive Go-Nogo procedure, we first trained 6 gerbils to perform an AM discrimination task (Nogo: 4 Hz; Go: 4.25-12 Hz) at a depth of 0 dB (re 100% depth, where

dB=20log[mod-depth-%/100%]). Animals were permitted to advance to the next depth if their false alarm (FA) rate was < 40% and their $d' > 1$. Once this criterion was reached, animals were trained on a shallower depth (-3dB). This training process was repeated for depths of -6, -9, -12, -15 and -18dB. Once animals were trained at each depth (0 to -18dB), their AM rate discrimination thresholds were obtained for all the depths.

Results: As the AM depth became shallower, animals required a great number of sessions to reach a criterion performance of $d'=1$ on the AM discrimination task. For example, 5 ± 1 sessions were required to move from -6 to -9 dB, while 9.67 ± 1.5 sessions were required to move from -12 to -15 dB. Each animal displayed a unique learning trajectory, with the best animals able to perform the task at a depth of -15 dB for an AM rate of 5 Hz. This depth is equivalent to the lowest thresholds that gerbils display on an AM depth detection task (Anbuhl et al., 2022).

Conclusions: These results suggest performance on challenging auditory perceptual tasks requires prolonged practice, with greater time needed to achieve criterion performance at harder depths, but ultimately leading to expertise.

T177. Auditory Peripersonal Space and the Looming Bias in Normal-Hearing Adults

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Background: This study investigates the relationship between the auditory peripersonal space (PPS) and the auditory looming bias (ALB) in normal-hearing (NH) adults. The PPS is the space immediately surrounding the body, characterized by an increased integration of information from multiple senses, including hearing. The ALB is a phenomenon in which approaching sounds attract involuntary attention, as signalled by increased behavioral and neural responses and phasic alertness, compared to receding sounds. Because detecting objects as they approach the PPS is vital for survival, looming sounds attract involuntary attention when their spatial characteristics are not originally monitored by the organism. This raises the question whether ALB is modulated by PPS or whether moving auditory objects are processed in a special way inside PPS regardless of whether they are looming. We hypothesized that (H1) for sounds of radial motion, looming sounds increase involuntary attention compared to receding sounds, affecting neural responses and phasic alertness, and (H2) for sounds of angular motion, there is an auditory PPS bias in which sounds in PPS increase involuntary attention compared to sounds in extrapersonal space (EPS), affecting neural responses and phasic alertness.

Methods: We employed a cued auditory target detection paradigm. NH participants detected the presence of tone pips (targets) preceded by moving sounds (cues). Four kinds of cues were used, based on the interaction of motion direction (radial or angular) and motion onset distance (EPS or PPS): looming into PPS, receding into EPS, rotating in PPS, rotating in EPS. Source intensities were controlled for by using low intensity and high intensity blocks. Behavioral data, electroencephalography (EEG), and skin conductance responses (SCR) were recorded to test the existence of an auditory PPS bias on target detection, its physiological and behavioral correlates, and its relationship to ALB.

Results: Target detection promptness, as well as SCRs and event-related potentials (ERPs) evoked by the moving cues will be analyzed in the next step. We contrast these three measures across radial (looming vs. receding) and angular (rotating in PPS vs. EPS) conditions to test H1 and H2, respectively. H1 would be corroborated if larger amplitude ERPs (particularly the P2 component reflecting motion detection) and SCR responses, and prompter target detection were found in reaction to looming sounds compared to receding sounds. H2 would be corroborated if a larger amplitude P2 ERP component and SCR response, and prompter target detection were found in reaction to angular sounds in PPS compared to angular sounds in EPS.

Conclusions: In conclusion, the present study tests whether auditory threat detection in NH listeners relies on the discrimination of motion direction (looming-receding difference) as well as on the monitoring of the sound source's distance relative to the listener (PPS-EPS demarcation).

T178. Ohm's Acoustic Law Revisited

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Background: Ohm's acoustic law suggests that perception of a complex tone depends only on the amplitudes of the tone's Fourier components while being invariant to the relative phase of these components. However, there is substantial evidence that long-term Fourier phase can affect perception, prompting many researchers to consider Ohm's acoustic law to be largely incomplete or incorrect. Long-term monaural phase has been shown to impact timbre, pitch salience, gap detection, and identification of fast temporal structure. Nonetheless, many modern machine learning systems use short-time Fourier (STF) amplitudes for speech or sound recognition while ignoring phase information. Here we revisit Ohm's acoustic law and study phase invariance in short-time Fourier and neurally-inspired, cochleogram sound representations using both modeling and psychoacoustic experiments with human listeners.

Methods: Using stimulus distance comparisons, we first model how short-time, amplitude-only representations might predict monaural effects previously considered violations of Ohm's acoustic law. We then use a vocoding approach to synthesize sounds with specific short-time amplitude representation but distinct phase structure. In studies with human listeners, we measure 1) quality ratings of waveforms vocoded with STF or cochlear representation amplitudes and 2) discriminability of vocoded waveforms from original sounds using an AXB paradigm.

Results: In our modeling, we find that changes in cochleogram amplitudes alone can often predict previously measured changes in perception of phase-manipulated speech and masking with phase-manipulated stimuli. Although these stimuli are designed by modifying the phase information under Fourier or STF filterbanks, these Fourier based modifications generally produce changes to the amplitudes obtained with a cochlear-like filterbank. In our psychoacoustic studies we find that, for speech and music, STF-vocoded waveforms are generally perceived as noisy or distorted. However, with cochleogram-inversion, these sounds can, in many cases, act as metamers that are near indistinguishable from clean sounds, despite having distinct waveforms and phase.

Conclusions: Together, these results suggest that a modified version of Ohm's acoustic law, with short-time phase invariance, may explain a wide range of perceptual results and may partially explain why machine learning methods do so well without phase information.

T179. Cell-Type-Aware Quality Control and Spatial Validation Improves Discovery in Single Nucleus RNA Sequencing of Heterogenous Inner Ear Tissues

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Background: Single-nuclei RNA sequencing (snRNAseq) uncovers the transcriptional landscape of complex tissues. Multiple quality control (QC) metrics are utilized to remove low quality droplets early in the snRNAseq pipeline. Often, one sample wide high and low threshold QC marks data removal. In heterogeneous samples, standard pipelines risk loss of biologically meaningful data to sample-wide QC thresholds. We propose a cell type aware QC pipeline to capture cell type specific QC metrics, balancing technical noise removal with biological data retention. Furthermore, snRNAseq data limits the ability to validate cell type annotations without further experimentation, highlighting the power of spatial transcriptomics as a validation method. Our pipeline addresses these challenges by integrating cell-aware QC filtering with robust spatial validation. We will demonstrate how heterogeneous samples benefit from this pipeline using the mature adult mouse inner ear, a diverse and highly specialized tissue.

Methods: This pipeline was implemented in R. Before starting QC filtering, a sample underwent Seurat preprocessing and clustering. These clusters were annotated for known cell-types. Outliers were identified on a per cell-type basis and flagged for potential removal. SoupX was then used to correct for ambient RNA and multiplets were detected with DoubletFinder. Droplets either unable to be annotated, identified as an outlier, or identified as a multiplet were removed from the dataset. The dataset was re-clustered and reannotated after droplet removal. To validate the results, we identified unique gene markers for each of our cell-type clusters and projected them onto a spatial transcriptomics dataset.

Results: Our results show improved retention of biologically meaningful droplets that would otherwise be lost when completing sample wide QC filtering thresholds. Initial cell-type annotation identified clusters for well characterized cell-types of the cochlea. Cell-type specific QC metrics were immediately evident for gene count, transcript count, and mitochondrial gene percentage. Sample wide threshold filtering resulted in a large proportion of particular cell-types to be lost while others were unaffected. Our cell-type specific QC thresholds resulted in outliers being identified throughout many cell-types of the sample. Once filtered, distinct clusters were evident and cluster-specific markers mapped to the spatial data set consistent with cell-type annotations.

Conclusions: Our cell-type aware QC pipeline overcomes current heterogenous tissue QC challenges. Biologically meaningful droplets with inherently unique cell-type specific QC metrics are retained and rich metadata flags provide detailed reasoning for data removal. These subsets allow for enhanced statistical power, detection of rare cell populations, and capture subtle biological variation in the biological and transcriptional landscape of the tissue.

Furthermore, integration with a spatially informed validation step offers a powerful method to confirm and contextualize cell-type specific expression patterns in their native spatial environments. While demonstrated in the mature mouse cochlea, this pipeline is broadly applicable to heterogeneous tissues and multi-modal transcriptomic studies.

T180. Effects of Mild Therapeutic Hypothermia on Human Cochlear Temperature

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Background: Hearing loss, driven by aging, genetics, noise, and ototoxic drugs, remains a major unmet medical challenge. While hearing aids and cochlear implants improve quality of life, they are costly, carry surgical risks, and cannot fully restore natural hearing. Among preventive strategies, mild therapeutic hypothermia (MTH) shows the strongest promise, protecting against cochlear trauma and noise-induced damage. However, current devices provide insufficient and uneven cooling, with vascular perfusion further limiting efficacy. We aim to develop optimized MTH approaches that incorporate human-specific anatomy and blood flow to achieve safe, uniform cochlear cooling for effective hearing preservation.

Methods: The study employed complementary computational and in vitro models to evaluate mild therapeutic hypothermia (MTH) for hearing preservation. A multiphysics head model was constructed from medical imaging data to simulate heat transfer in the temporal bone and cochlea while incorporating vascular perfusion dynamics to capture the influence of blood flow on cooling. Predictions from this model were validated using a physiologically realistic in vitro human head phantom fabricated with tissue-mimicking materials and embedded vascular flow, enabling controlled benchmarking of MTH protocols. Together, these platforms provided a clinically relevant framework for evaluating cooling duration, spatial temperature distribution, and safety thresholds in human anatomy.

Results: Computational simulations demonstrated that vascular perfusion strongly influenced intracochlear temperature, with carotid blood flow maintaining near-core temperatures unless explicitly accounted for in cooling strategies. Optimized modeling predicted that uniform cochlear hypothermia (31–33 °C) could be sustained for up to 120 minutes when cooling load and placement were tailored to human anatomy. Validation in the in vitro head phantom confirmed these predictions, showing that perfusion-mimicking flow reduced superficial overcooling and produced more uniform temperature distributions compared to passive gel-based systems. Together, these findings established the feasibility of achieving safe and effective cochlear cooling using human-specific anatomical and vascular considerations.

Conclusions: These studies demonstrate that integrating vascular dynamics into computational models and validating them with physiologically realistic in vitro platforms enables accurate prediction and optimization of cochlear cooling. The combined modeling and experimental platforms not only advance hearing preservation research but also provide versatile tools for future exploration of auditory interventions.

T181. A Computational Model of Light Propagation and Thermoviscoacoustic Impacts for Optical Stimulation

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Background: Optical stimulation is emerging as a promising method for applications such as infrared neural stimulation, cochlear implants, optogenetics, photopharmacology, and photobiomodulation therapy. However, understanding the interaction of light with tissues is critical to ensuring both safety and efficacy of these methods. Light absorption, scattering, and subsequent bio-physical effects during optical stimulation, particularly thermal and viscoacoustic responses, remain underexplored. This work presents a computational framework to evaluate how optical stimulation propagates within cochlear structures and its implications for tissue safety and auditory perception.

Methods: Optical propagation and mode distribution were analyzed using COMSOL Multiphysics simulations of step-index multimode fibers under bending and side-polishing conditions, enabling assessment of spatial multiplexing for multi-site stimulation. To quantify safety, Monte Carlo ray tracing and bio-heat transfer models were used to estimate local temperature changes in cochlear tissue exposed to pulsed near-infrared light. Thermoviscoacoustic responses were then derived from the transient heat load using Pennes' bio-heat equation and validated against in vitro temperature recordings near the fiber tip. Cumulative Equivalent Minutes at 43 °C (CEM43°C) were calculated to evaluate both thermal and thermoviscous safety limits under relevant stimulation conditions.

Results: COMSOL modeling of step-index multimode fibers revealed stable mode confinement with negligible bending losses, supporting their use for curved cochlear geometries. Furthermore, spatial mode diversity enables the potential for multiplexed stimulation, as simulations showed distinct leakage patterns from higher-order modes when side-polished, suggesting selective out-coupling at different cochlear sites. Based on simulation results, the temperature rise was determined to be < 2 °C under pulsed stimulation at wavelengths of 700–1100 nm. By contrast, stimulation at 400–600 nm produced higher temperature increases, up to 6 °C, due to the greater absorption of light in this range. The safe cumulative equivalent minutes at 43 °C (CEM43°C) for cochlear tissue corresponds to a damage threshold of 1.9 min. Each treatment should therefore be modeled separately, accounting for the specific timeline and waveform, to accurately assess thermal safety.

Conclusions: Our computational framework provides an integrated perspective on optical, thermal, and viscoacoustic processes due to optical stimulation in cochlea. The findings indicate that appropriately optimized optical stimulation can achieve spatially confined activation while maintaining tissue safety. These insights open pathways to exploring combined neural and viscoacoustic mechanisms of hearing restoration and preservation. This model serves as a foundation for guiding experimental validation and the development of next-generation cochlear implant technologies.

T182. Redesigning the Fluid Jet: A Non-Contact Stimulator With Stiff Probe Speed

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Background: The opening of mechano-electrical transduction (MET) channels in hair cells is the first step in hearing. These channels, located at the tips of shorter stereocilia rows, are activated when the hair bundle is deflected toward the tallest row. The primary in vitro stimulation techniques to study MET currents are stiff probes (glass pipettes mounted on piezo stacks) and fluid jets (oil-driven nozzles, typically powered by piezo buzzers). Stiff probes offer very high temporal precision (> 10 kHz) but require direct contact, which can alter bundle mechanics and limit stereocilia visualization. Fluid jets avoid contact and allow symmetric push/pull deflections, but conventional designs are limited to > 1 kHz, far below acoustic frequencies and rates that make it difficult to separate between adaptation processes. Our aim was to overcome this limitation by developing a high-speed fluid jet actuator.

Methods: Using CAD Software, we engineered a redesigned fluid jet with reduced oil volume, a reshaped oil chamber, and a shortened pipette outlet. We replaced the traditional piezo buzzer with a high-frequency piezo stack actuator. Device performance was assessed in rat (P8-P11) inner and outer hair cells using whole-cell patch clamp recordings and high-speed imaging (> 10 kHz). To validate actuator speed independently of bundle mechanics, we also measured the deflection of fibers by our new design and a more traditional fluid jet.

Results: The new design substantially improved the frequency response of fluid jet stimulation. Whereas conventional buzzer-based jets rarely exceed 1 kHz, our actuator consistently drove bundle motion above 5 kHz, reaching up to 20 kHz in some cases. Our design was also capable of producing step actuations, like IX and paired-pulse stimulations, where adaptation of the MET current was observed. Fiber deflection experiments confirmed both faster rise times and larger displacements compared to traditional designs. Importantly, these gains were achieved without sacrificing the inherent advantages of fluid jets, including non-contact stimulation, symmetric push-pull capability, and unobstructed optical access for imaging.

Conclusions: This is, to our knowledge, the first fluid jet stimulator capable of achieving acoustic-range frequencies that rival stiff probe performance. By combining the contact-free benefits of fluid jets with temporal precision in the kilohertz range, this new tool enables experiments that were previously impossible. It allows MET channel kinetics, bundle viscoelasticity, and membrane mechanics to be studied under conditions closer to those in vivo, with implications for understanding normal auditory function and its disruption in hearing loss.

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T183. Development, Validation and Applications of a Model for Predicting Audiogram Changes in Adults Undergoing Cisplatin Chemoradiotherapy

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Background: Ototoxic hearing loss is a common and debilitating adverse effect in adults undergoing cisplatin-based chemotherapy or chemoradiotherapy, particularly among cancer populations with preexisting vulnerabilities. Current prediction approaches are limited because they typically estimate risk only with respect to one classification system, rather than providing full audiogram-level forecasts. This lack of precision reduces opportunities for early detection and tailored counseling. Developing multivariate prediction tools capable of forecasting complete audiograms would allow clinicians to anticipate and mitigate disability, while enabling more comprehensive ototoxicity monitoring.

Methods: We developed and externally validated the VA Hearing Loss Evaluation and Risk Screener (VA HEARS), a Bayesian multivariate linear mixed-effects model designed to predict individualized audiogram outcomes following cisplatin treatment. The model incorporates pre-treatment thresholds, patient age, cisplatin and head/neck radiation exposures. Removing cisplatin variables permits the model to be used for ongoing treatment-phase ototoxicity monitoring. Data for development were drawn from a single Veterans Affairs medical center, including patients treated between 2010 and 2022 for training, and patients treated between 2019 and 2024 for validation. A total of 131 male Veterans aged 42–80 years (mean 65.2), with cancers primarily of the head/neck (38.1%), lung (38.1%), or oropharynx (12.9%), contributed up to eight audiograms each. Cumulative doses averaged 62.3 mg/m² (range 25–100 mg/m²), with 47% receiving concurrent cochlear radiation. The primary outcomes were predicted 0.5–8 kHz thresholds in both ears. Secondary measures were detection of clinically significant shifts classified using American Speech-Language-Hearing Association (ASHA) and Common Terminology Criteria for Adverse Events (CTCAE) standards.

Results: In predicting post-treatment thresholds, the VA HEARS model achieved precision within clinical audiology error ranges, with root mean squared errors of 4.3–8.5 dB in the training cohort and 5.2–10.6 dB in validation (higher error at higher test frequencies). Classification accuracy for detecting significant shifts was moderate-to-strong, with area under the curve values of 0.78 for both ASHA and CTCAE in validation data. Audiometric forecasts further demonstrated treatment-dose interactions: at a cumulative cisplatin dose of 280 mg/m², predicted 8 kHz thresholds increased by an average of 12 dB in the presence of cochlear radiation compared to 8 dB without radiation. These findings underscore the model's utility both for pre-treatment counseling and for ongoing monitoring during cancer treatment.

Conclusions: The VA HEARS model represents the first externally validated framework for forecasting cisplatin-induced audiogram changes across full frequency ranges in adult patients. By producing individualized predictions and harmonizing multiple ototoxicity classification standards simultaneously, the model provides a flexible, clinically relevant approach to hearing loss forecasting. It is adaptable for both baseline risk assessment and longitudinal monitoring. Incorporating such predictive tools into oncology care may enhance counseling, enable early interventions, and reduce long-term disability in patients treated with ototoxic agents.

T184. Interaction of the Effects of Acoustic Transients and Narrow-Band Sustained Sound Input on Responses in the Inferior Colliculus

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Background: Acoustic transients, including rapid onsets are a salient feature of many natural sounds including speech and adventitious sounds, like rustling. These abrupt increases in sound pressure cue scene parsing, event detection, and attention, and are processed by specialized auditory mechanisms tuned for temporal precision. For example, octopus cells (OCs) in the cochlear nucleus are especially sensitive to these sharp onsets and provide precisely timed inhibitory input to the inferior colliculus (IC) via the ventral nucleus of the lateral lemniscus¹. Despite this well-defined anatomical pathway and functional importance of rapid onsets in object-segmentation and streaming², the role of this inhibitory pathway, and more broadly how responses to transient and sustained sound components interact in the IC, remains unclear.

Methods: To investigate the nature of this interaction, we constructed gaussian-gated tone complexes at the Gabor limit, with specified bandwidth and center frequency, which we call ‘clicklets’ (i.e. clicks limited in frequency analogous to wavelets, which are waves limited in time). We examined the impact on tone-evoked responses in IC of parametrically varied clicklets across varying temporal offsets, reflecting the abrupt onset of one sound object and the sustained component of a second object, using Neuropixels recordings in awake, head-fixed mice.

Results: A PSTH-based linear model indicated a sublinear integration of tone and click responses, suggesting suppressive interaction when stimuli are combined. Modulation of tone responses was strongest when clicks and tones were presented simultaneously, consistent with rapid, precisely timed inhibition. Additionally, rapid-onset stimulation differentially modulates IC neuron responses depending on neurons’ receptive fields and the spectral content of the clicklets, with the weakest modulation occurring when the clicklets center frequency closely matches the neurons’ preferred frequency and strongest at the complementary frequencies.

Conclusions: Our findings suggest that rapid acoustic onsets engage precisely timed circuit interactions in the IC, likely involving OCs, leading to sublinear integration and suppression of tone responses. This interaction is strongest at minimal delays, suggesting a possible mechanism for enhancing temporal contrast. In addition, the response modulation observed at complementary frequencies may contribute to sharpening spectral contrast. Together, these spectro-temporal enhancements could provide a neural substrate for auditory object streaming and segmentation.

T185. GFP Immunohistochemistry of the Cochlea Following Microwave Decalcification of the Temporal Bone

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Background: Gene therapy is currently being investigated as a potential tool to prevent hearing loss using a modified adenoviral vector containing the Green Fluorescent Protein gene (GFP) as a reporter gene. In order to confirm cellular transfection of the ear via GFP immunohistochemistry (IHC), decalcification of the cochlea must be performed prior to tissue sectioning and staining. While EDTA is the decalcification method of choice for IHC, this process can take up to 13 weeks for cynomolgus macaque temporal bone. We show here how using microwaves in combination with EDTA can significantly shorten decalcification time without any negative effects on GFP IHC.

Methods: Temporal bones from cynomolgus macaques and GFP transgenic mice (C57BL/6-Tg(UBC-GFP)30Scha/J, Jackson Laboratory) were collected and were fixed in formalin for approximately 48 hours before being decalcified in 10% EDTA alone or with microwaves (25 to 30 Celsius degrees, 500 Watts). After decalcification, the tissues were embedded in paraffin and step-sectioned to obtain mid-modiolar sections of the cochlea which were either stained with H and E or underwent GFP IHC using a polyclonal rabbit anti-GFP antibody.

Results: Histologically, all sections decalcified with the microwaves were morphologically undistinguishable from the sections decalcified with EDTA alone. In the GFP transgenic mouse, GFP IHC was equivalent between temporal bones decalcified with microwaves and those decalcified with EDTA alone. For cynomolgus macaques, full temporal bone decalcification (including entire middle and inner ear) took as little as 26 days while decalcification of temporal bone containing the inner ear only was 11 to 17 days. Thus, microwave decalcification significantly reduced the decalcification times and did not appear to negatively affect GFP IHC in GFP transgenic mice or quality of H and E sections in either species.

Conclusions: The combined use of EDTA and microwaves for decalcification of cynomolgus macaques temporal bone greatly decreases decalcification times and appears to be a promising decalcification method for use in gene therapy studies.

T186. Histopathological Evaluation of the Vestibular System

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Background: Many chemicals including numerous drugs are known ototoxic agents. However, inner ear evaluation, in particular the vestibular system, is not routinely performed in preclinical safety assessment. We show here the normal microscopic anatomy of the vestibule and semi-circular canals as well as some typical drug-related findings.

Methods: Temporal bones from cynomolgus macaques, guinea pigs (including some animals administered gentamicin via middle ear catheterization) and mice were collected and were fixed and decalcified in a solution of formalin, picric acid and formic acid and then transferred to a refrigerator set to maintain 4°C or were fixed in formalin for approximately 48 hours and decalcified in EDTA. After decalcification, the tissues were embedded in paraffin, step-sectioned (75 to 150 µm intervals), mounted on glass slides, and stained with H and E.

Results: Many important substructures of the vestibular system, including the utricle and the saccule along with their macula as well as the ampulla at the base of each semicircular canal, were readily found and examined on routine H and E sections. In guinea pigs administered gentamicin, degenerative and/or atrophic changes were noted in the macula and ampulla.

Conclusions: The numerous substructures of the vestibular part of the inner ear are readily available for histopathological evaluation. Sensory hair cells are susceptible to injury from ototoxic agents and should not be overlooked during preclinical safety assessment.

T187. Use of Select Questionnaires to Evaluate Gait Disturbances Under Simulated Conditions of Intoxication

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Background: When individuals are confronted by police for driving erratically (swerving, going too fast or too slow, going through stop signs or red lights, etc.), they are evaluated to determine if they were operating their motor vehicle under the influence of alcohol or drugs. These procedures fall under the rubric of “field sobriety testing (FST);” a collection of behavioral tasks to assess balance skills, coordination abilities, and reflexes.

While interpretation of FST is key to this assessment, these results are qualitative in nature, potentially limiting their effectiveness in identifying impairments. This research was intended to enhance detection of potential abnormalities by studying various temporospatial gait parameters in a controlled laboratory setting.

Methods: In 40 healthy adults ranging in age from 19 to 66 years, two conditions were evaluated to determine gait-related effects: 1) having individuals ambulate on a computerized walkway (GAITRite Walkway System) under normal conditions without goggles, and 2) having individuals ambulate on a computerized walkway under identical conditions but using “drunk-buster goggles” to simulate intoxication. For each task, 10 separate temporospatial gait parameters were evaluated (distance, ambulation time, velocity, step count, cadence, step length, stride length, stride velocity, single-support time, and double support time). A 2-way (2 x 5) repeated measures analysis-of-variance (ANOVA) was used to evaluate the effects of intoxication and condition where 5 different tasks were evaluated:

1. Walking in a straight line with eyes open and with hands by your side,
2. Walking in a straight line with eyes open and arms extended to each side,
3. Walking in a straight line with eyes open and hands by your side while reciting the alphabet,
4. Walking in a straight line with eyes open and hands by your side while counting backwards by 3 and,
5. Walking in a straight line, taking 5 to 9 steps, then turning on one foot, and returning in the opposite direction.

Results: Statistical main effects of intoxication and condition were observed for all individuals and tasks. There was no condition by intoxication interaction for any of the variables studied. We also considered a more comprehensive multivariate analysis to further explore these relationships.

Conclusions: This research was intended to educate police on intoxication-based walking patterns using quantitative assessment strategies. These findings expand current behavioral assessment strategies using gait as the dependent variable.

T188. Evaluating Temporospatial Gait Parameters Under Simulated Conditions of Intoxication

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Background: When individuals are confronted by police for driving erratically (swerving, going too fast or too slow, going through stop signs or red lights, etc.), they are evaluated to determine if they were operating their motor vehicle under the influence of alcohol or drugs. These procedures fall under the rubric of “field sobriety testing (FST);” a collection of behavioral tasks to assess balance skills, coordination abilities, and reflexes.

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Conclusions: This research was intended to educate police on intoxication-based walking patterns using quantitative assessment strategies. These findings expand current behavioral assessment strategies using gait as the dependent variable.

T189. Immediate and Long-Term Effects of Repeated Noise Exposure on Vestibular Short-Latency Evoked Potentials and Vestibular Sensory Epithelia

Marie Anderson¹, David Bauer¹, Shelby Powers², Lisa Kabara¹, Ariane Kanicki¹, Richard Altschuler¹, Courtney Stewart^{*2}

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Background: It has been established that noise exposure dose-dependently damages the inner ear, resulting in hearing and vestibular loss as well as impaired mobility. We have previously

demonstrated transient effects of a single 110dB SPL noise exposure on hearing and vestibular function assessed with auditory brainstem response (ABR) and vestibular short-latency evoked potential (VsEP). More recently, shorter (3-hour) broadband noise exposures have been repeated every 2-weeks over an 8-week period (4 exposures total), resulting in slower walking speed that recovers within weeks after the final noise exposure. After recovery, deficits are still present when visual information is not available. In this condition, rats that appear to recover from repeated noise exposure still have slower balance beam crossing speeds than control rats or their own crossing times when visual information is available. This suggests increased dependence on vision when vestibular information is compromised. In the current study, we ask how this noise exposure affects VsEP responses over time, and how vestibular sensory epithelia are affected 1-month and 1-year after the final noise exposure, at 10- and 21-months of age, respectively.

Methods: Between 7-9 months of age, rats were exposed to 4, 3-hour 110dB SPL broadband noise exposures, each spaced 2-weeks apart. VsEP and ABR were assessed at baseline, and 1-, 7-, and 28-days after the final noise exposure, or 1-year after the final noise exposure, at 21-months of age. Ears were collected from noise exposed or control rats 28-days after the final noise exposure at 10-months of age or 1-year after the final noise exposure at 21-months of age. Total and calyx-only terminals, and synaptic ribbons were imaged in whole-mounted vestibular sensory epithelia using a Leica Stellaris 5 confocal microscope and quantified in Imaris 10.2.

Results: Although repeated noise exposure had a small impact 28-days after noise exposure, a greater impact is seen 1-year after the final noise exposure. This may suggest that small noise exposures that minimally impact vestibular responses after weeks of recovery seem to accelerate aging, resulting in smaller VsEP responses than controls 1-year after the final noise exposure. More interestingly, fewer synaptic ribbons were identified and fewer calyceal terminals expressed calretinin 1-year after exposure to repeated noise versus age-matched controls, suggesting that even relatively small amounts of noise exposure, when repeated, persistently damage the vestibular periphery.

Conclusions: These results highlight the importance of limiting noise exposures, even before measurable hearing or vestibular loss occurs. Even small noise exposures that result in minor changes in hearing or vestibular function may accelerate aging processes resulting in impaired inner ear function and reduced mobility.

T190. Age-Related Changes in Vestibular and Auditory Function in the Fischer 344-Brown Norway F1 Hybrid Rat

Shelby Powers¹, David Bauer², Marie Anderson², Lisa Kabara², Richard Altschuler², Courtney Stewart*¹

¹*VA Ann Arbor Healthcare System*, ²*University of Michigan Kresge Hearing Research Institute*

Background: Age-related vestibular decline is a complex issue, but environmental factors such as noise exposure may contribute to vestibular dysfunction, particularly in human subjects since environmental noise exposure is unavoidable in our world. Traffic noise, sporting events, concerts, restaurants, and clubs, just to name a few, are sources of significant noise exposure and hearing protection during recreational activities is frequently under-utilized. The rat model provides an interesting perspective into natural aging of the vestibular periphery, as laboratory animals are generally held in highly regulated, low noise environments. The current study

characterizes declining vestibular and hearing function related to aging using the Fischer 344-Brown Norway F1 Hybrid (F344BNF1) rat strain. Results of this work will provide normative data for future studies of the long-term effects of noise exposure in the F344BNF1 rat.

Methods: F344BNF1 rats were obtained from the National Institute on Aging (NIA) Rodent Colony at approximately 6, 12, 18, 21, 24, 28, and up to 31 months of age. Vestibular short-latency evoked potential (VsEP) and pure tone auditory brainstem response (ABR; 1.5, 4, 8 kHz) was assessed in each age group. Auditory and vestibular thresholds were compared with one-way ANOVA followed by a multiple comparisons test. Input-output functions for wave I response characteristics to each pure-tone ABR frequency were analyzed individually for all age groups using two-way ANOVA followed by a multiple comparisons test. Input-output functions for VsEP response characteristics (P1N1 and P2N2 amplitude and onset latency) were individually assessed across all stimulus intensities and all age groups using two-way ANOVA followed by a multiple comparisons test.

Results: ABR thresholds remain stable in most rats at 18 months of age; however, VsEP thresholds are elevated by this time. Input-output functions suggest that although ABR thresholds are stable at 18-months of age, response amplitudes are smaller by this time in response to all pure tone frequencies tested. In addition to the observation of elevated VsEP thresholds, VsEP responses to small stimuli were also smaller in rats older than 18-months of age.

Conclusions: Our results indicate that vestibular function declines prior to auditory function in the F344BNF1 rat. It is possible that some of the differences observed in the current work are related to body weight and other factors not accounted for in the current work. Future work will examine age-related changes in vestibular sensory epithelia to see if changes in the inner ear correlate with the observations of the present work.

T191. Monitoring Head and Trunk Position With a 9-Axis Sensor Integrated Into a Video-Oculography System

Makoto Hashimoto*¹, Okinaka Yosuke², Junko Tsuda², Kazuma Sugahara¹

¹*Yamaguchi University Graduate School of Medicine*, ²*Yamaguchi University*

Background: Infrared CCD cameras are essential for clinical examination of the vestibular system. Video-oculography (VOG) devices allow accurate recording of human eye movements. We developed an advanced VOG and image filing system for real-time three-dimensional analysis of nystagmus and created a high-quality video Frenzel goggle system (yVOG-Glass).

Methods: Video images were recorded using a high-resolution Frenzel camera at 60 frames per second with 640 × 480-pixel resolution. Horizontal and vertical components were analyzed by calculating the pupil center coordinates. Torsional components were analyzed by overlaying successive iris patterns and determining the angle of maximal correlation. Head position was simultaneously monitored using a 9-axis sensor.

Results: Real-time and accurate measurements of horizontal, vertical, and torsional eye movements were obtained. Quantitative analysis, including slow-phase velocity of nystagmus, was performed automatically. The 9-axis sensor enabled precise monitoring of head position, allowing synchronized recording of eye and head movements.

Conclusions: The yVOG-Glass system enables real-time quantitative three-dimensional analysis of nystagmus using high-quality video Frenzel recordings. Integration of a 9-axis sensor allows monitoring of head and trunk position, which is expected to be useful for vestibular assessment and rehabilitation.

T192. High-Salt Intake Exacerbates Endolymphatic Hydrops and Alters Aldosterone Regulation in a Ménière's Disease Animal Model

Makoto Kinoshita^{*1}, Akinobu Kakigi², Yuki Katsutani¹, Teru Kamogashira¹, Kento Koda¹, Kentaro Ichijo¹, Chisato Fujimoto¹, Shinji Yamashita¹, Kenji Kondo¹

¹*The University of Tokyo, Graduate School of Medicine*, ²*Kochi Medical School*

Background: Ménière's disease (MD) is characterized by episodic vertigo and hearing fluctuation, with endolymphatic hydrops (EH) as its pathological hallmark. Salt restriction is commonly recommended to manage MD, but its underlying mechanism remains unclear. The endolymphatic sac (ES), especially its extraosseous segment, plays a crucial role in endolymph absorption and expresses aldosterone (Aldo)-sensitive sodium transporters. This study aimed to clarify how ES dysfunction and high salt intake interact to influence EH, vestibular function, and Aldo regulation.

Methods: Using a guinea pig model, ES dysfunction was induced by electrical cauterization, followed by either normal-salt (0.41% NaCl) or high-salt (2.0% NaCl) diet. Animals were divided into four groups (n=4 each): Sham-Normal, Sham-Na, ES-Normal, and ES-Na. Vestibular evoked potential (VsEP) thresholds and plasma Aldo were measured at weeks 4 and 6 post-surgery. At week 6, EH was quantified via optical coherence tomography (OCT), assessing extension ratio of Reissner's membrane length (ER-L) and increase ratio of scala media area (IR-A). Statistical analysis was performed using two-way ANOVA and post hoc tests.

Results: OCT analysis revealed that ES-Na groups exhibited significantly greater ER-L (48.2%) and IR-A (72.5%) than other groups, indicating a synergistic effect between ES dysfunction and high-salt intake on EH formation. VsEP thresholds were significantly elevated by both ES lesion and high-salt diet (main effects: $p < 0.05$), but without interaction, suggesting additive vestibular impairment. While plasma osmolality remained stable across all groups, Aldo concentration showed a significant interaction: Aldo was markedly elevated in the ES-Normal group (159.0 ± 44.8 pg/mL) but suppressed under high-salt conditions regardless of ES status. This suggests that in the context of ES damage, dietary salt loading disrupts the compensatory renin–angiotensin–aldosterone system (RAAS)-driven Aldo response, potentially exacerbating endolymph imbalance.

Conclusions: ES dysfunction significantly increases susceptibility to high salt intake, promoting EH and disrupting Aldo-mediated regulatory mechanisms. The data support sodium restriction in MD patients, particularly those with ES damage. These findings also point to RAAS modulation as a potential therapeutic strategy. Future studies incorporating molecular profiling and long-term intervention are warranted to further elucidate inner ear fluid regulation mechanisms.

T193. Role of Pou4f3 in Utricular Hair Cells Survival and Vestibular Function in the Mouse Models of Pou4f3 Deletion at Adult Age

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¹*Southern Illinois University School of Medicine*, ²*Head and Neck Surgery, University of Mississippi Medical Center*

Background: Germline knockout and mutation studies have demonstrated the crucial role of the transcription factor Pou4f3 in hair cell (HC) differentiation and survival in the mammalian cochlea during embryonic development. Additionally, Pou4f3 germline knockout mice display impaired vestibular function, deficits in differentiation of type I HCs and loss of vestibular HCs. However, Pou4f3's function in postnatal vestibular HCs remains poorly understood. Our preliminary findings suggest selective loss of type I utricular HCs after deletion of Pou4f3 from both HC types at birth. Using CreER lines that favor either type I or type II HCs we further investigated the impact of Pou4f3 deletion at 8 weeks of age on utricular HCs and vestibular function.

Methods: Using CreER/loxP mice, we deleted Pou4f3 from adult utricular HCs using Fbxo2-CreER:Pou4f3loxP/loxP or Atoh1-CreER:Pou4f3loxP/loxP mice to preferentially target type I or type II HCs, respectively. Mice received two injections of tamoxifen (~24 hours apart) at 8 weeks of age. Among these injected litters, Cre-negative (Pou4f3loxP/loxP) and Cre-only littermate pups served as controls. We also examined Fbxo2-CreER:Pou4f3+/loxP and Atoh1-CreER:Pou4f3+/loxP pups to study potential Pou4f3 haploinsufficiency. Otolith function was assessed by measuring the translational vestibulo-ocular reflex (tVOR) at 0.2-2 Hz at 6 weeks and 6 months after Pou4f3 deletion, followed by sample collection. For the Fbxo2-CreER line, utricular samples were analyzed at the 6 months post-deletion timepoint only. Utricles were dissected from the temporal bones and immunostained for myosin VIIa and Sox2. Type I and type II HCs were counted in the striolar and extrastriolar regions of the utricles.

Results: Preliminary comparison of the number of type I or type II HCs within the utricular region displayed no significant difference across time when Pou4f3 was deleted using Atoh1-CreER mice. Additionally, within each timepoint and utricular region, there were no significant differences in the number of type I or II HCs across genotypes. After targeted deletion of Pou4f3 predominantly from type I HCs (using Fbxo2-CreER mice), there was a significant reduction in the number of type I HCs in Pou4f3 knockout utricles compared to Cre-only controls in all three utricular regions analyzed. The number of type II HCs remained unchanged when compared across genotypes. The most prominent effect of Pou4f3 deletion on tVOR responses appeared to be an increase in gain for the Atoh1-CreER:Pou4f3loxP/loxP and Atoh1-CreER:Pou4f3+/loxP mice as compared to controls at 6 weeks after tamoxifen. At 6 months post-tamoxifen, there were no such dramatic differences in gain or phase, for either the Fbxo2-CreER or Atoh1-CreER mice, suggesting minimal behavioral effects of the loss of extrastriolar type I HCs or the loss of Pou4f3 from either HC type.

Conclusions: Preliminary data suggest that type I HCs are dependent on Pou4f3 for their survival in the adult utricle, while type II HCs are not.

T194. Postural Improvements With Prolonged Noisy Galvanic Vestibular Stimulation Correlate With Short-Term Effects Over a Wider a Range of Current Intensities

Chisato Fujimoto*¹, Takuya Kawahara², Yayoi Kikkawa¹, Makoto Kinoshita³, Teru Kamogashira³, Mineko Oka¹, Kentaro Ichijo³, Kenji Kondo³, Shinichi Iwasaki⁴

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Background: The optimal intensities of noisy galvanic vestibular stimulation (nGVS) to improve postural stability vary daily, but frequent measurement is clinically challenging. This study aimed to investigate the relationship between the ameliorating effects of short-term nGVS at various intensities on postural stability and those of the prolonged nGVS at optimal intensity performed on different days. Specifically, we sought to determine which current intensities of short-term nGVS were effective in the group that showed improvement with prolonged nGVS.

Methods: Patients with peripheral vestibulopathy underwent closed-eye center of pressure measurements for 30 seconds without nGVS and with nGVS at 100-2000 μ A to determine optimal intensity (the current yielding the greatest velocity improvement). Prolonged nGVS was then applied at this intensity. Patients with at least 10% improvement in mean velocity change during 3 hours of stimulation versus placebo were classified as the "improvement" group, while others formed the "no improvement" group. The per-cent change in velocity from no nGVS at each intensity was compared between the two groups.

Results: The improvement group (n = 9) had a significantly greater improvement than the no improvement group (n = 30) at 100 μ A and 1700 μ A ($p < 0.05$). The improvement group tended to show improvement over a wider range of current intensities of short-term nGVS.

Conclusions: The ameliorating effects of prolonged nGVS on postural control were associated with those of short-term nGVS over a wider range of current intensities.

T195. Vestibular Aqueduct Hypoplasia Identifies Semicircular Canal Dehiscence Patients Who Progress to Menière's Disease

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Background: Semicircular canal dehiscence (SCD) and vestibular aqueduct (VA) hypoplasia are developmental anomalies that may be associated with distinct inner ear syndromes—SCD syndrome (SCDS) and Menière's disease (MD). We previously found a high prevalence of radiological SCD in the MD subgroup with VA hypoplasia (MD-hp), suggesting a common developmental origin and a possible clinical "overlap" syndrome. Here, we investigated the reciprocal association by assessing the prevalence and significance of VA hypoplasia in patients with computed tomography (CT)-confirmed SCD.

Methods: We retrospectively reviewed temporal bone CT scans from 219 ears of 173 patients (mean age 53.5 ± 16.6 years; 54.3% female) evaluated for suspected SCD at a tertiary referral center. SCD diagnosis was based on axial, coronal, Stenvers and Pöschl views. SCD cases associated with the superior petrosal sinus were excluded. VA morphology was assessed using

the angular trajectory of the vestibular aqueduct (ATVA) marker; an ATVA $> 140^\circ$ was considered hypoplastic. Clinical records were screened for MD diagnosis according to current Bárány criteria.

Results: VA hypoplasia was identified in 4/173 SCD patients (2.3%), all of them exhibiting superior SCD (SSCD), representing a ~46-fold increase compared to the estimated 0.05% prevalence of MD-hp in the general population. All four cases demonstrated an atypical SCD location overlying the posterior limb of the superior semicircular canal, spatially adjacent to the hypoplastic VA. Moreover, all four patients were diagnosed with MD ipsilateral to the side of SCD/VA hypoplasia.

Conclusions: The co-occurrence of SSCD of the posterior limb and VA hypoplasia in a distinct patient group supports a shared developmental anomaly of the retrolabyrinthine temporal bone, as both structures arise from the same ossification center. The presence of VA hypoplasia identifies SCD patients on a progressive trajectory toward MD with irreversible hearing and balance loss. Recognition of this CT pattern may allow earlier diagnosis, counseling, and tailored monitoring.

T196. Engineering a Noninvasive, Affordable 3D Vestibulo-Ocular Reflex Evaluation Platform

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Background: Vestibular dysfunction affects nearly 40% of individuals in the United States during their lifetime and is a major contributor to falls, disability, and reduced quality of life. Accurate assessment of the vestibulo-ocular reflex (VOR) is essential for diagnosing vestibular deficits, guiding therapy, and monitoring recovery. Current gold-standard approaches, such as scleral coils and high-end video-oculography (VOG), are costly, technically demanding, and often uncomfortable for patients. Even commercial video head impulse testing (vHIT) systems remain expensive, limited to two-dimensional measurements, and prone to artifacts.

Methods: We propose a system integrating markerless pupil tracking with gyroscope-based head motion monitoring. Preliminary studies demonstrate the ability of this approach to capture 3D eye and head kinematics at high temporal resolution. The project is organized into three phases: (1) development of a robust markerless VOG platform and algorithms for 3D pupil tracking validated against scleral coil data; (2) integration of gyroscope-based head motion monitoring and synchronization of eye-head data against a calibrated reference system; and (3) evaluation of system performance in detecting unilateral and bilateral vestibular dysfunction in human subjects, benchmarked against clinical standards.

Results: Preliminary findings suggest that precise 3D VOR gain can be measured noninvasively and that the system is sensitive to pathological vestibular conditions.

Conclusions: This work aims to establish the first validated, affordable, and painless 3D VOR assessment tool. The technology has the potential to democratize vestibular testing, enable longitudinal monitoring, and advance understanding of balance disorders, with broad implications for clinical care and rehabilitation.

T197. Restoration of Otolith Function and Gait Stability Using a Vestibular Implant: An in Vivo Pilot Study

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Background: Bilateral vestibular hypofunction (BVH) causes visual motion sensitivity, postural instability, and gait disturbances, markedly reducing quality of life. Current vestibular rehabilitation alleviates symptoms but does not restore vestibular function. This study evaluated the effects of chronic otolith-targeted stimulation via a prototype artificial vestibular implant in a BVH mouse model.

Methods: Bilateral vestibular lesions were induced in 30 CBA/J mice, followed by unilateral implant placement in the left ear. Fourteen mice were included in the final analysis and assigned to three groups: no stimulation (n=4), 0.5 V stimulation (n=5), and 1.0 V stimulation (n=5). At 2 weeks post-surgery, otolith-ocular reflex (OOR), vestibulo-ocular reflex (VOR), and gait parameters were assessed.

Results: In the 1.0 V stimulation group, vertical eye rotation in the OOR improved to levels comparable to controls, whereas VOR gain remained reduced across all groups. Behavioral testing revealed significantly reduced trunk deviation in the 1.0 V group, similar to controls. Gait analysis demonstrated a significantly narrower base of support in the stimulation groups; however, stride length and walking speed showed no significant improvement.

Conclusions: Chronic otolith stimulation using a prototype vestibular implant partially restored otolith function and enhanced postural stability in BVH mice. Further optimization of stimulation parameters and electrode design may provide the foundation for future clinical application of vestibular implants.

T198. Assessment of Sensory Evoked Potentials in Balance Deficit Animals: Electrophysiological Signals Correlating to Behavioral Alterations and Vestibular Organ Changes Within Acute Ototoxic Iminodipropionitrile Exposure

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Background: Vestibular deficits in the aging population have been increasing as the life expectancy of individuals have steadily improved due to technological and lifestyle advancements. Such causes of balance disorders may vary in degree wherein the causative agent of such ailments have proven to be either physical, chemical, environmental, or genetic. With such varying factors in inflicting disorientation, a means of providing a reproducible animal model with quantifiable characteristics have been proven challenging. Vestibular sensory-evoked potentials (VsEP) allow detection of electrical responses to the brain based on the stimuli originating from the vestibular organs. In addition to conventional evaluation of behavioral discrepancies such as open field and balance tests, VsEP can target either the function of utricle

and saccule in accordance with spatial recognition. This setup can be correlated to morphological changes which can be detected through exposure of the vestibular apparatuses.

Methods: The relevant study provided includes acute ototoxic damage of Sprague Dawley rats with 3-3'Iminodipropionitrile to induce damage similar to aminoglycoside antibiotics. With observance within a 7-day timeframe to minimize pain of the animal, behavioral tests were conducted via balancing on a rotating platform, latency detection of animal righting reflexes, and curling behavior which detects spatial orientation of the animal. Additional tests were analyzed using open field exploration of the animal to detect any hyperactivity or immobility of the animal during different timepoints of the experiment. In addition, vestibular sensory-evoked potentials were checked under anesthesia to evaluate vestibular organ responses of the animal to correlate any changes relevant to its behavioral disposition.

Results: Using 1,000 mg/kg IDPN administration, relevant behavioral discrepancies have been detected at Day 3 through incapability of balance and changes with mobility as seen through different behavioral patterns. Interestingly, we see discrepancies on VsEP only at Day 7 which indicates that the underlying causes of behavioral changes may be damaging to other organs and vestibular changes were more prominent at the last point of the experiment. Utricular changes in the hair cell morphology and counts were evident to be diminished at Day 7 whereas histological staining of the utricle and saccule showed deterioration of both hair cells as well as otoconia after IDPN administration.

Conclusions: Preliminary results on the discrepancies of behavior were immediately observed before seeing changes in vestibular to brain responses of the animal. This is to show that early changes on the behavior of animals may also be contributed to other factors. In addition, further experiments may be attributed such as molecular mechanisms and further analysis of other electrical signals may show better translation between VsEP and its corresponding behaviors.

T199. Peripheral Vestibular Dysfunction in Mouse Models of Autism

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Background: It is well documented that autism spectrum disorder (ASD) is associated with vestibular dysfunction, resulting in postural instability, gait dysfunction, and impaired gaze. These impairments are typically attributed to abnormalities in central neural pathways (Stins and Emck, 2018; Butera et al., 2023; Miski et al., 2022), while the role of peripheral sensors—specifically those in the inner ear and vestibular nerve—remains largely unexplored. Despite their potential significance, the vestibular periphery is rarely assessed for dysfunction in ASD. In this study, we examined peripheral vestibular function in two established ASD mouse models—Shank3b knockout (KO) and Cntnap2 KO mice—and explored potential correlations between vestibular deficits and behavioral performance in balance and spatial memory tasks.

Methods: We assessed peripheral vestibular function in male and female Shank3 and Cntnap2 KO mice (heterozygous and homozygous) aged 3–8 months. Age- and sex-matched wild-type (WT) littermates served as controls. We evaluated (1) the vestibular nerve via vestibular sensory evoked potentials (VsEP), (2) vestibular function by contact righting reflex, and (3) spatial memory by the Y-maze and Barnes maze tests.

Results: Both Shank3 and Cntnap2 KO mice showed impaired peripheral vestibular function, indicated by reduced VsEP P1N1 amplitudes, particularly in males (Shank3 KO: -23.6%, Cntnap2 KO: -54.8%). Females showed milder (Cntnap2 KO: -33.7%) or no deficits (Shank3 KO). These changes may reflect disrupted potassium-dependent non-quantal (NQ) and glutamatergic quantal (Q) transmission in the vestibular periphery. Supporting this, ~40% of male Shank3 KO and > 50% of Cntnap2 KO mice of both sexes showed abnormal contact righting reflexes. Both models also exhibited cognitive impairments: male Shank3 KOs had deficits in Barnes maze learning, while Cntnap2 KOs showed spatial memory deficits in the Y-maze across sexes. A correlation was observed for Cntnap2 KO mice between decreased VsEP responses and increased time to right ($m = -77.79$, $r^2 = 0.34$, $p = 0.003$).

Conclusions: Our preliminary findings indicate sex-specific peripheral vestibular dysfunction in Shank3 and Cntnap2 KO mouse models, with more pronounced deficits observed in males. The data suggest impairments in both NQ and Q transmission in the vestibular periphery. While correlations between peripheral vestibular function and spatial memory performance were modest, they point to potential relationships that should be explored further.

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T200. A New Noise Exposure Model Reveals Motor Deficits Linked to Ongoing Changes in Vestibular Responses in Adult Rats

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Background: Falls are the leading cause of injury-related deaths among adults ≥ 65 years. The vestibular system plays a significant role in gait, postural control, and balance. Damage to this system can be a major contributor to imbalance and impaired motor control, increasing fall risk. Studies of motor function have demonstrated noise-induced motor impairments in neonates, animal models of Ménière's disease, and in animals with abolished vestibular short latency evoked potentials (VsEPs). To determine the consequences of noise-induced decreases in VsEP responses on motor function, rotarod performance was evaluated over time.

Methods: Sprague-Dawley rats ($n=4$) were assessed for motor coordination using an accelerating rotarod (5 to 45 rpm over 60s) under light and dark conditions. VsEP responses were compared at baseline, one hour, 3-, and 7-days post noise exposure (NE). Repetitive jerk stimulation (3,300g/s) was delivered through attachment of the skull to a mechanical shaker, which delivered linear acceleration (6,000 jerks in three intervals of 2,000 jerks each) in the naso-occipital plane. After baseline rotarod performance was assessed, each rat was exposed to a 120 dB SPL 1.5 kHz-centered 3-octave band noise for 4-hours free-field in wire mesh cages. Motor function was then reassessed 3- and 10-days post-NE. The primary outcome was reported as fall latency (fall time minus start time).

Results: After NE, changes in VsEP latency and amplitude were observed at each time point. One hour after NE, latency was increased in P2 and P3 of the VsEP. By 3- and 7-days post-NE, P1 amplitudes increased while P3 amplitudes decreased, and both P2 and P3 continued to exhibit increased latency shifts. For rotarod performance in the light, NE significantly reduced the

latency to fall from 10.75 seconds (baseline) to 4.75 seconds (3-day post-NE; $p=0.033$) and 4.25 seconds (10-day post-NE; $p=0.049$). Interestingly, although not significant, performance in the dark tended to increase latency to fall from 10.25 seconds (baseline) to 17 seconds (3-day post-NE; $p > 0.05$) and 13.75 seconds (10-day post-NE; $p > 0.05$).

Conclusions: A novel NE model in adult animals appears to affect the vestibular system, producing a permanent threshold shift that increases response times and reduces synchrony. Additional changes continued to occur over time. Motor impairment was evident under light conditions but not in the dark. Visual–vestibular conflict may contribute to mismatched sensory information, impairing performance in the light, whereas in the dark, rats may rely more on proprioceptive and tactile cues, reducing sensory conflict and allowing performance to stabilize or even improve. Peripheral and central deficits (early and late VsEP waves) in irregular vestibular afferent function observed by 3-days post-NE appear to correlate with motor performance, highlighting the need for future studies to determine histological correlates and whether these changes also affect other vestibular-related function such as cognition or skilled walking.

T201. Characterizing Differences in Endolymphatic Hydrops Signatures Among Meniere's Disease Patients With and Without Migraine

Yoshiyuki Sasano^{*1}, Fumihiro Mochizuki², Yusuke Ito², Erin Williams¹, Izumi Koizuka², Manabu Komori², Michael Hoffer¹

¹*University of Miami Miller School of Medicine*, ²*St. Marianna University School of Medicine*

Background: Differentiating Meniere's disease (MD) from vestibular migraine (VM) is challenging due to overlapping clinical features. Migraine is frequently comorbid with MD, and this coexistence may complicate diagnosis and interpretation of inner ear imaging. While endolymphatic hydrops (EH) has been studied in MD and VM separately, comparative imaging data for MD patients with and without migraine remain limited.

Methods: We evaluated the distribution and laterality of EH using endolymphatic contrast-enhanced MRI (HYbriD of Reversed image of Positive endolymph signal and native image of positive perilymph signal, or HYDROPS) in patients with definite MD, with or without comorbid migraine.

We retrospectively reviewed 78 patients with definite MD who underwent HYDROPS between October 2021 and March 2022. Patients were classified into two groups: MD only ($n=56$) and MD with comorbid migraine (MD+Mg, $n=22$). Migraine was diagnosed according to ICHD-3 criteria. The degree of EH (negative, mild, or significant) was assessed separately in the inner ear, the cochlea and vestibule of both ears by three otolaryngologists specialized in vestibular disorders and two head and neck radiologists. Chi-square tests were used to compare the distribution of endolymphatic hydrops (unilateral vs. bilateral), and to compare the severity of hydrops (significant, mild, or negative) among the three locations of interest. Bonferroni correction was applied to correct for Type I error due to multiple comparisons.

Results: In the MD group, the affected ear showed significantly higher rates of significant EH compared to the unaffected ear ($p < 0.01$), which was consistent across the inner ear, cochlea and vestibule. Conversely, in the MD+Mg group, we found no significant differences in EH

distribution between the affected and unaffected ears. Interestingly, the MD+Mg group showed a significantly higher frequency of significant EH in the unaffected cochlea ($p < 0.01$). This trend was concordant across regions, appearing in the inner ear ($p < 0.025$) and in the vestibule ($p < 0.05$). No significant differences were observed between the MD and MD+Mg groups in terms of hydrops severity in any region of the affected ear. Finally, bilateral hydrops was significantly more frequent in the MD+Mg group than in the MD group among all regions investigated ($p < 0.05$).

Conclusions: Our findings suggest that patients with MD and comorbid migraine demonstrate a distinct EH distribution pattern, characterized by symmetrical or bilateral EH and significant hydrops in the clinically unaffected ear. This raises the possibility that migraine-related pathophysiological mechanisms may contribute to EH formation. In clinical settings, the presence of bilateral EH on HYDROPS MRI in suspected MD cases should prompt consideration of comorbid migraine, in addition to bilateral MD or asymptomatic hydrops.

T202. Balance Function in STAT1 Heterozygous Mice

Shotaro Harada^{*1}, Tomoko Makishima¹

¹*University of Texas Medical Branch*

Background: Signal transducer and activator of transcription 1 (Stat1) knockout (KO) mice with moderate immunodeficiency have been used frequently in viral infectious disease models. Our goal was to determine the baseline balance function in Stat1 KO heterozygous mice to assess whether they are suitable for use in vestibular function studies.

Methods: Male and female wild type (WT) 129Sv/Ev mice ($n=5$ each), male and female Stat1 heterozygous mice ($n=5$ each), and male and female Stat1 KO mice ($n=5$ each) at 3 months of age were compared. Motor coordination and balance were assessed using the rotarod and balance beam tests. The rotarod started at 4rpm accelerating 1 rpm every 8 seconds to 40 rpm. Latency, distance traveled, and rotations per minute (RPM) were recorded during this test. The experiment lasted three days with a total of nine trials per mouse. The balance beam test consisted of a 100 cm long flat surface with widths of 5 mm and 10 mm. Time to traverse the beam, number of paw slips, and weight of the mouse were recorded at 3 months of age. Each mouse was tested on both widths three times, totaling six trials per mouse.

Results: On the rotarod, Stat1 KO mice (Latency; 92.6 ± 21.9 s, Distance; 40.7 ± 13.1 cm, RPM; 9.5 ± 1.9) outperformed Stat1 heterozygous mice (Latency: 85.8 ± 18.0 s, Distance: 37.0 ± 13.7 cm, RPM: 8.9 ± 1.6) and WT mice (Latency: 88.6 ± 32.2 s, Distance: 38.8 ± 22.4 cm, RPM: 9.2 ± 2.8).

On the balance beam, Stat1 heterozygous mice showed better balance function (5mm; 4.5 ± 1.4 cm/s, 10mm; 6.1 ± 2.1 cm/s) than Stat1 KO mice (5mm; 2.7 ± 0.9 cm/s, 10mm; 4.7 ± 2.3 cm/s) or WT mice (5mm; 2.3 ± 0.2 cm/s, 10mm; 6.0 ± 2.7 cm/s).

Conclusions: The results of this study suggested that there is difference in motor coordination and balance between the Stat1 KO or Stat1 heterozygous mice and WT mice. Future studies will include comparison between males and females as well as the addition of auditory evaluation (auditory brainstem response, distortion product otoacoustic emission) and vestibular evaluation with eye movement analysis (vestibular ocular reflex).

We conclude that both Stat1 heterozygous mice and Stat1 KO mice have slightly different balance function characteristics compared to Stat1 WT mice and that the usage in infectious disease models should be done with caution and inclusion of appropriate control groups.

T203. Directional Change of Nystagmus During Vertigo Attacks Predicts the Progression of Cochlear Endolymphatic Hydrops and Poor Hearing Prognosis in Meniere's Disease

Hiroki Takeda*¹, Kayoko Higashi-Shingai¹, Takao Imai², Takashi Sato¹, Yoshiyuki Ozono¹, Ryohei Oya¹, Yuya Ueno³, Hidenori Inohara¹

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Background: Spontaneous nystagmus is observed during vertigo attacks in patients with Meniere's disease (MD). The mechanism of direction and directional change of nystagmus during vertigo attacks has been discussed in relation to the pathophysiology of MD. We developed the Portable Eye Movement Recorder (PEMR), a self-recording device for eye movements to capture nystagmus during vertigo attacks at home. We examined the relationship between the direction and directional change of nystagmus during vertigo attacks, and audio-vestibular function and inner ear imaging findings in patients with MD.

Methods: We enrolled 77 patients with unilateral definite MD (31 males and 46 females; age: 20–84 years with median age of 60 years) and analyzed nystagmus recorded using PEMR at home. Each patient was instructed to record eye movements using PEMR on four occasions: at the onset of a vertigo attack, at the end of the attack, 1 hour after the end, and the day after the attack. Directional change of nystagmus was defined as the direction of nystagmus at the onset of the attack being opposite to that of any subsequent nystagmus. We examined the relationship between the direction and directional change of nystagmus during attacks, and age, sex, disease duration, hearing thresholds, vestibular functions and the volume of endolymphatic hydrops in the cochlea and vestibule measured using gadolinium-enhanced MRI.

Results: At the onset of vertigo attacks of MD, 53 patients showed nystagmus beating toward the affected side (irritative nystagmus, IN) and 18 showed nystagmus beating toward the healthy side (paralytic nystagmus, PN), while 6 showed no nystagmus. There was no association between the direction of nystagmus at the onset of vertigo attacks and age, sex, disease duration, audio-vestibular function and inner ear imaging findings. Among 39 patients who recorded on four occasions, directional change of nystagmus was observed in 29 patients (IN to PN: 22, PN to IN: 7), while direction-fixed IN was observed in 10 patients. None of the patients showed direction-fixed PN. Directional change of nystagmus during vertigo attacks of MD was significantly associated with the volume of cochlear, but not vestibular, endolymphatic hydrops (odds ratio: 1.22). Hearing thresholds 1–3 years after the recording in patients with directional change of nystagmus during vertigo attacks were significantly increased compared to those without directional change, although there was no significant difference in hearing thresholds before the recording between patients with and without directional change of nystagmus.

Conclusions: These findings suggest that the progression of cochlear endolymphatic hydrops induces vertigo attacks with directional change of nystagmus, leading to poor prognosis of

hearing loss in patients with MD. Directional change of nystagmus during vertigo attacks is a predictor of the progression of cochlear endolymphatic hydrops and poor hearing prognosis in MD.

Mini Podium 3: Inner Ear: Damage and Protection of Hair Cells

3:00 p.m. - 4:00 p.m.

Meeting Room 209ABC, Floor 2

Moderators: Cathy Sung and Weishen Alice Liang

Multimic Analysis Reveals Cell-Type Specific Mechanisms of Cisplatin-Induced Ototoxicity

3:00 p.m. - 3:15 p.m.

Deanne Nixie Miao^{*1}, John Pham¹, Alana Lamont¹, Emilia Luca², Alain Dabdoub², Britt Drogemoller¹

¹*University of Manitoba*, ²*Sunnybrook Research Institute/ University of Toronto*

Background: Cisplatin causes ototoxicity in up to 80% of patients, with children facing a three-fold higher risk. Genetics plays a major role in cisplatin-induced ototoxicity (CIO). We hypothesize that genetic risk variants modulate cisplatin-induced gene expression changes in the cochlea, contributing to CIO. To identify the genes, pathways, and cell types involved in CIO, we profiled gene expression changes in the cochlea using single-nuclei (sn) RNA sequencing (RNA-seq) and snATAC-seq.

Methods: Postnatal day-6 CBA/CaJ mice received intraperitoneal injections of 3mg/kg cisplatin (treatment, n=6) or saline (control, n=6). Four-hours post cisplatin administration, mice were euthanized, and cochlear ducts were dissected. This timepoint was selected to capture early cisplatin-induced gene expression and chromatin accessibility changes prior to cell death. Nuclei were isolated using the 10X Genomics Nuclei Isolation Kit, followed by library preparation using the 10X Genomics Multiome Kit. Sequencing was performed on the NovaSeq X Plus. Multimic data were processed using Cell Ranger ARC and analyzed using Seurat and Signac. Differential gene expression was assessed with DESeq2 and enriched biological pathways were identified with fgsea. Finally, CellChat was applied to compare intercellular communication networks between groups.

Results: Differential gene expression analysis (DESeq2: $P_{adj} < 0.05$, $|\log_2FC| \geq 2$) revealed significant cell-type-specific gene expression differences between cisplatin- and saline-treated mice. Increased expression of *Clu*, *Col9a3* [basal stria vascularis (SV)], *Cst3*, *S100a1*, *Mia* [outer hair cells (OHCs)], *Col12a1* [pre-osteoblasts (pOBs)], and *Col9a2* [type I neurons (T1N)] was observed in cisplatin-treated mice, while *Acp5* (T1N) was the only downregulated gene relative to controls. Enriched pathways included mitochondrial energy metabolism (SV), autophagy and stress response (OHCs), proteasome or ubiquitin-mediated degradation (pOBs), and RNA splicing, lipid metabolism and axon maintenance (T1Ns). These transcriptional changes highlight distinct cell-type-specific pathways through which cisplatin may affect cochlear function and

integrity. CellChat analysis revealed that cisplatin reduced both the number (control=7,634; treatment=6,410) and overall interaction strength (control=195; treatment=152) of cell-cell interactions.

Conclusions: Our study identified key cochlear genes, pathways, and cell types that are involved in CIO. The SV, OHCs, and TINs are confirmed as vulnerable targets, while pOBs emerge as a new contributor. In the SV of cisplatin-treated mice, we observed increased expression of *Clu*, with inhibition/knockout of this gene reported to enhance the efficacy of cisplatin and reduce kanamycin- and furosemide-induced ototoxicity. Further, *Col9a3*, *Col12a1* and *Col9a2* are collagen-encoding genes whose dysregulation may weaken extracellular matrix integrity and increase CIO susceptibility. Cisplatin's ability to bind collagen and release platinum slowly may further prolong cochlear exposure and exacerbate this damage. Future work will integrate these murine multiomic datasets with human genomic CIO studies to help define clinically relevant risk factors for this adverse drug reaction.

Single-Nucleus RNA-Seq Profiling of Cisplatin-Induced Hearing Loss

3:15 p.m. - 3:30 p.m.

Amanda Bonczkowski^{*1}, Franz Gareza¹, Erica Sadler¹, Katharine Fernandez¹, Rafal Olszewski¹, Michael Hoa¹, Tessa Sanders¹, Matthew Kelley¹, Mark Warchol², Ran Elkon³, Ronna Hertzano¹, Cathy Yea Won Sung¹, Lisa Cunningham¹

¹NIDCD, ²Washington University School of Medicine, ³Tel Aviv University

Background: Cisplatin is a widely used anti-cancer drug with significant ototoxic effects, resulting in permanent hearing loss. Upon entering the cochlea, cisplatin causes death of mechanosensory hair cells (HCs) that convert sound into neuronal signals. However, the mechanisms underlying the death of HCs in response to cisplatin are not well understood. In this study, using a previously developed clinically relevant mouse model of cisplatin-induced ototoxicity, we performed single-nucleus RNA-Sequencing (snRNA-Seq) to examine HC-specific transcriptional changes associated with hearing loss in adult mice. Specifically, we analyzed transcriptional changes of HCs when hearing loss is evident.

Methods: Our mouse model involves three cycles of cisplatin treatment, each cycle consisting of once-daily cisplatin injection for four days followed by a ten-day recovery period. In this model, hearing loss begins during the cycle 2 recovery period and progressively worsens throughout the third cycle. Cochleae were harvested from saline-treated control mice as well as mice treated with cisplatin at two time points – after the cycle 3 cisplatin injection period and after the cycle 3 recovery period. snRNA-seq was performed using the 10X Genomics platform. The snRNA-Seq datasets were analyzed using the Seurat R package to cluster and annotate cells according to their expression profiles and known gene markers. The expression profile of the HC cluster was analyzed using differential gene expression analysis, followed by k-means clustering, to then apply gene set enrichment analysis (GSEA), and Gene Ontology (GO) terms to understand transcriptional changes in groups of genes associated with cisplatin-induced hearing loss.

Results: A total of 3609 OHC and 1195 IHC were analyzed across the 3 timepoints. There were significantly more differentially expressed genes for OHC than IHC when comparing the control timepoint to the other timepoints. GO terms comparing the control group to cycle 3 cisplatin

injection group and control to cycle 3 recovery group uncovered multiple mechanisms related to cisplatin-induced hearing loss. In both comparisons, HCs display changes related to stereocilia movement and ion transport, which may reflect disruptions in sensory transduction and cellular homeostasis. Additionally, the activation of wound healing and DNA repair processes suggests that the cells are attempting to repair cisplatin-induced damage. In the cycle 3 cisplatin injection period, GO terms associated with mitochondrial respiratory chain and ATP production were observed, indicating that cisplatin is impacting cellular metabolism and energy production. Consistent with observed HC loss, GO terms associated with apoptosis were seen in cycle 3 injection and cycle 3 recovery groups.

Conclusions: Our results demonstrate that transcriptomic changes in cochlear HCs are associated with apoptosis, cellular homeostasis, and DNA repair. Further work will validate the impacts of these processes and investigate transcriptional responses to cisplatin in other cochlear cell types.

Lysosomal Calcium Release Drives Hair Cell Survival After Aminoglycoside Uptake

3:30 p.m. - 3:45 p.m.

Francisco Barros-Becker^{*1}, Ananya Cholkar¹, Patricia Wu¹, Tor Linbo¹, David Raible¹

¹*University of Washington*

Background: Mechanosensory hair cells in the inner ear are complex and highly specialized cells that fulfill a pivotal role in hearing and balance. Hair cells are susceptible to ototoxic damage from therapeutic agents, like the widely used aminoglycoside (AG) family of antibiotics. Despite their known ototoxic effects, AGs, like neomycin and gentamicin, are still considered standard treatment for patients. Our group has explored the multiple adverse effects of AG on hair cells of the zebrafish lateral line, including the distinct hair cell death patterns that different AGs generate. During uptake, neomycin accumulates in the cytoplasm of hair cells generating acute death of the hair cell. Other aminoglycosides, like gentamicin or G418, accumulate mostly in lysosomes within hair cells, and they generate delayed hair cell death. These observations led us to hypothesize that vesicular compartmentalization and lysosomal function could be influencing a delayed hair cell death. Our ultimate goal is to understand the lysosomal pathways that participate in delayed hair cell death and survival.

Methods: In order to understand how lysosomal function is able to influence hair cell death, we aim to study AG delivery into the lysosome, followed by the effects of perturbation to the lysosomal compartment. Using transgenic zebrafish lines and fluorescently labeled AGs we follow the autophagic delivery of AGs into the lysosome. Moreover, using lysosomotropic drugs and ion channel agonists we dissected specific pathways that influence the delayed hair cell death process. These effects will be assessed by performing dose response curves, as well as live imaging and automated image analysis.

Results: Our results support the previous observation that most G418 accumulation happens in late endosomes/lysosomes, labeled by Rab7. Autophagosome, but not early endosomal, colocalization of G418 indicate autophagocytosis as main lysosomal loading pathway. Lysosomal stress generated by GPN is able to protect hair cells against G418, but not against neomycin. Similarly, TPC2-A1-N, a Two Pore Channel 2 (TPC2) calcium agonist, is also able to

protect against G418, but not against neomycin. This protection is only present when TPC2-A1-N is added before G418, suggesting that there are other mechanisms involved necessary for protection. Alternatively, sodium released from the lysosome through TPC2 does not protect against AG toxicity.

Conclusions: Our data suggests autophagocytosis as the main mechanisms of accumulation G418 into lysosomes. Calcium release, by GPN or TPC2-A1-N protects hair cells against G418 toxicity, but not neomycin exposure. This protective effect is specific to calcium, as stimulation of other ion release doesn't change susceptibility. Overall, our data suggests that lysosomes are a key factor in the delayed, but not acute, hair cell death process, and calcium plays an important role during protection.

Cross-Species Insights Into Mitochondrial Mechanisms of Delayed Hearing Loss Following Cisplatin Chemotherapy

3:45 p.m. - 4:00 p.m.

Neva Bergemann^{*1}, Elayna Malak², Mona Jawad³, Josef Trapani², Mark A. Rutherford³, Lavinia Sheets¹

¹*Washington University School of Medicine in St. Louis*, ²*Amherst College*, ³*Washington University St. Louis*

Background: Cisplatin is a widely used chemotherapeutic that causes permanent and progressive hearing loss that can occur years following treatment. Late-onset hearing loss suggests patients are more vulnerable to noise-induced damage and environmental insults after cisplatin treatment. While cisplatin damages cochlear mechanosensory hair cells, the subcellular mechanisms driving their vulnerability remain incompletely understood. As a result, there are limited therapeutic options that also preserve cisplatin's efficacy. Previous work in zebrafish has shown that cisplatin accumulates in and damages hair cell mitochondria. To further advance our understanding of hair cell mitochondrial dysregulation related to cisplatin, we utilized two complementary model systems: zebrafish lateral line hair cells and the murine cochlea. Zebrafish larvae provide ease of in-vivo investigation of hair cell injury due to their larval transparency and superficial location of hair cell bundles (neuromasts) along their lateral line, whereas mouse models allow for mechanistic and functional validation in a mammalian cochlear system.

Methods: Zebrafish at 6 days post-fertilization (dpf) were treated with low (30 μ M, 100 μ M) and mid- (300 μ M) dose cisplatin for 2 hours followed by a 24-hour recovery period. Quantitative confocal live-imaging was performed to assess hair cell mechanotransduction, mitochondrial morphology, and mitochondrial membrane potential using the fluorescent vital dyes FM1-43 and TMRE, respectively. To further investigate disruption of hair cell mitochondrial homeostasis, mitochondrial biogenesis was evaluated using fluorescent in situ hybridization and immunohistochemistry. Parallel studies in mice, mirroring the Fernandez et al. cisplatin mouse model, are investigating cisplatin-induced hearing loss from a high dose (4 mg/kg) that induces hearing loss in addition to a lower dose (1 mg/kg) where hearing loss is absent.

Results: In zebrafish, surviving hair cells exhibited altered mitochondrial morphology and mitochondrial depolarization following low dose cisplatin, yet also display normal

mechanotransduction at low doses. Preliminary data suggests increased mitochondrial biogenesis in cisplatin treated hair cells, despite evidence that mitochondrial count decreases in response to cisplatin, suggesting a disruption in the balance between biogenesis and mitophagy. In mice, ongoing work is comparing mitochondrial dynamics across these low and high dose cisplatin groups with functional outcomes measured by auditory brainstem responses (ABR). We predict that with moderate synaptopathic noise exposure, the low dose cisplatin group will acquire more severe hearing loss than controls due to increased vulnerability within the cochlea.

Conclusions: Cumulatively, these results suggest that hair cells which survive cisplatin display normal mechanotransduction, yet significant mitochondrial dysfunction and cellular stress. These findings underscore the importance of considering hair cell mitochondrial health in the management of cisplatin ototoxicity and support the translational value of combining zebrafish and mammalian systems to identify mechanisms and therapeutic strategies for patient care.

t 4: Auditory Brainstem: Structure and Function

3:00 p.m. - 4:00 p.m.

Ballroom B, Floor 3

Moderators: Luberson Joseph and Ruili Xie

Diverse Dendrites of the Rat Superior Paraolivary Nucleus

3:00 p.m. - 3:15 p.m.

Randy Kulesza*¹

¹*Lake Erie College of Osteopathic Medicine*

Background: The rat superior paraolivary nucleus (SPON) is composed of a homogenous population of GABAergic neurons that respond to pure tone and amplitude modulated tones with offset spikes. The rat SPON sends major projections to the ipsilateral central nucleus of the inferior colliculus and ventral division of the medial geniculate. The offset response of SPON neurons appears to be formed through an inhibitory rebound mechanism driven by dense, somatic glycinergic inputs from the ipsilateral the medial nucleus of the trapezoid body (MNTB). Tract tracing studies indicate that SPON neurons receive excitatory input from octopus cells in the contralateral ventral cochlear nucleus as well as additional inputs from the cochlear nuclei and medial superior olive. Several studies have revealed dendrites of ventrally situated SPON neurons extending from the nucleus into the ventral nucleus of the trapezoid body (VNTB). It is unclear what proportion of SPON neurons extend dendrites into the VNTB and further how inputs onto these dendrites might shape responses on SPON neurons.

Methods: We undertook an investigation of the rat SPON to elucidate the location and proportion of neurons that extend dendrites beyond the nucleus in an effort to better understand the repertoire of inputs to the SPON and how these might impact responses and function of the SPON. We used immunohistochemistry for microtubule-associated protein (MAP2) in three animals to examine general features of SPON dendrites. Additionally, we used Golgi or Golgi-Cox impregnations in 20 animals to reconstruct complete dendritic arbors of individual SPON neurons.

Results: Study of MAP2 immunolabeling revealed several large bundles of MAP2-positive dendritic profiles along the rostrocaudal extent of the SPON extending ventrally into the VNTB, MNTB and medial superior olive. Reconstruction of 78 SPON neurons from impregnated tissue sections revealed a more elaborate extension of SPON dendrites. Specifically, 85% of SPON neurons in the ventral and central aspect of the nucleus extended dendrites into the VNTB. Additionally, many SPON neurons extended dendrites ventrally into the MNTB, dorsal medial wedge and MSO. In addition, we found that many SPON neurons extended dendrites dorsally into the reticular formation and laterally into the lateral superior olive.

Conclusions: The rat SPON extends dendrites along the ventral aspect of the brainstem into the trapezoid body and nearly all nuclei in the superior olivary complex. Inputs onto the long-reaching dendrites may play an important role in keeping SPON neurons primed for incoming stimuli and contribute to SPON neurons coding sound offset with high precision.

Lateral Olivocochlear Neurons Integrate Multimodal Inputs to Regulate Cochlear Function

3:15 p.m. - 3:30 p.m.

Gabriel Romero*¹, Adrienne Cheung¹, Katelyn Boulanger¹, Stephanie Rouse¹, Lisa Goodrich¹

¹*Harvard Medical School*

Background: Hearing requires dynamic regulation by the central nervous system, which provides efferent feedback to adjust cochlear sensitivity across changing acoustic environments and behavioral contexts. Within this system, lateral olivocochlear (LOC) neurons in the superior olivary complex form the primary efferent projection to the cochlea, where they are positioned to regulate auditory nerve excitability and fine-tune auditory processing. Previous work has shown that LOCs upregulate multiple signaling molecules following acoustic trauma, providing a flexible feedback signal to the cochlea. Analysis of LOC depleted animals further suggests a role in regulating auditory nerve activity following damage. However, the circuits and stimuli driving LOC activity remain incomplete, creating a fundamental gap in our understanding of auditory feedback.

Methods: To reveal the inputs to LOCs, we employed a monosynaptic tracing strategy using pseudotyped rabies virus selectively targeted to LOCs. Retrograde helper virus was delivered via the posterior semicircular canal to restrict expression of TVA and rabies glycoprotein to LOCs, limiting EnvA-pseudotyped rabies infection to LOCs and their monosynaptic inputs. Whole-brains were cleared and registered to the Allen atlas for analysis. In parallel, we used FosTRAP2 mice crossed with a TdTomato reporter line to genetically tag LOCs activated by specific stimuli. Mice received 4-hydroxytamoxifen during acoustic stimuli, including noise and pure tones, followed by histological identification of TRAP+ LOC somata and axons.

Results: Rabies tracing revealed robust labeling of auditory brainstem nuclei—including cochlear nucleus and medial nuclei of the trapezoid body—as well as higher-order auditory regions, including the inferior colliculus and auditory cortex. Unexpectedly, inputs were also detected from limbic structures, including the ventral tegmental area and hippocampus, supporting that arousal and stress-related circuits directly engage LOCs. Complementary FosTRAP2 experiments demonstrated that LOCs are strongly and tonotopically activated by acoustic stimuli, with low-frequency broadband noise engaging the largest population. Labeled

LOC axons spanned large domains of the cochlea, consistent with the idea that efferent feedback has broad effects on cochlear responses.

Conclusions: Together, these approaches begin to elucidate the types of stimuli that recruit LOCs and the potential impact of multiple brain areas on their activity. The convergence of auditory and limbic inputs suggests that LOCs integrate multimodal signals to flexibly adjust auditory nerve excitability, possibly even before sound is detected. These findings provide a foundation for understanding how efferent control of the cochlea is engaged during trauma, arousal, or stress, with implications for hearing protection and adaptive auditory processing. Future work will extend this approach with in vivo physiology to link specific states and stimuli to LOCs at the single-unit level.

Take-away: LOCs are active modulators of cochlear output that are dynamically engaged by a variety of inputs and stimuli, positioning them as a critical node for integrating sensory and brain-state information in hearing.

The Properties of Intrinsic Lateral Olivocochlear Feedback to the Cochlea Throughout Maturation, Ageing, and Hearing Dysfunction

3:00 p.m. - 3:45 p.m.

Adam Carlton*¹

¹*University of Sheffield*

Background: Inner hair cells within the cochlea are solely responsible for the transduction of all perceivable sound information, which is then transmitted to the brain by type I spiral ganglion neurons (SGNs). The activity of these SGNs is modulated by three components arising from the brainstem and, of these, the intrinsic lateral olivocochlear (iLOC) feedback from the lateral superior olive (LSO) has been shown to possess remarkable plasticity. For example, iLOC feedback is cholinergic and excitatory to SGNs by default, but can dynamically upregulate inhibitory neurotransmitters based on sound experience. These neurons also appear to form direct synaptic contacts onto inner hair cells during hearing dysfunction caused by ageing or genetically disrupted sound transduction via Myo7a knock-out. The function of this rewiring is unclear, but recapitulates the pre-hearing configuration where inner hair cells receive direct cholinergic input. However, the role and properties of iLOC feedback remains poorly understood, but is hypothesized to assist with sound localisation, hearing in noise, and resistance to sound induced damage.

Methods: To investigate the underlying biophysical characteristics and better understand iLOC feedback across the lifespan (6 to > 500 days of age), whole-cell patch clamp electrophysiology was employed at body temperature on C57BL6/N or Myo7xMyo15Cre conditional knock-out mouse brainstem slices containing the LSO followed by fixation, immunolabelling, and biocytin based cell tracing.

Results: It was found that mature iLOC neurons possessed the same current threshold as pre-hearing neurons, and lacked the previously reported enhancement of spontaneous activity. However, through the downregulation of IA and upregulation of slower outward potassium current, mature iLOC neurons required less charge input to trigger an action potential and could fire at a higher rate with a smaller initial delay relative to pre-hearing neurons. Strikingly, despite

how the aged cochlea recapitulates the immature cochlea in terms of iLOC innervation, aged (> 1 year) neurons did not show any biophysical reversion, and instead showed further enhanced non-IA outward potassium currents resulting in even higher firing rates with faster spikes. Interestingly, while robust inner hair cell reinnervation is a common feature, sound transduction dysfunction caused by post-hearing Myo7a deletion did not shift the biophysics of iLOC neurons in any way.

Conclusions: This study reveals that throughout life the amplification power of iLOC neurons is steadily upregulated, possible to compensate for peripheral deficits in sound sensitivity with ageing. It also indicates that while inner hair cell reinnervation with ageing and dysfunction somewhat recapitulates the immature system, this is not paired with any similar biophysical reversions. Reinnervation also appears to not be the trigger for ageing-like iLOC characteristics within the LSO, and seems entirely independent. Taken together, this work provides new insights of how iLOC feedback adapts to optimally regulate hearing throughout life and during hearing dysfunction.

Disruption of Endocannabinoid Signaling Impairs Synaptic Transmission and Auditory Processing in the Cb1r Knockout Mice

3:45 p.m. - 4:00 p.m.

Jorge Contreras*¹, Jun Hee Kim¹

¹*University of Michigan*

Background: Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by deficits in social communication, impaired speech, and repetitive behaviors. Importantly, most individuals with ASD exhibit auditory processing abnormalities during early development. Recent clinical studies suggest that cannabidiol treatment may alleviate core ASD symptoms. However, the cellular mechanisms linking auditory dysfunction to the endocannabinoid system (ECS) remain poorly understood.

Methods: In this study, we hypothesized that activity-dependent activation of cannabinoid receptor 1 (CB1R) is essential for synaptic plasticity and auditory brainstem circuit maturation. Using Cb1r knockout mice, we investigated the role of ECS signaling in modulating neurotransmitter release and short-term plasticity at auditory brainstem synapses. In vivo auditory assessments, including auditory brainstem response (ABR) and acoustic startle reflex (ASR) tests, revealed significantly reduced startle responses in Cb1r knockout mice during postnatal development, despite normal auditory thresholds, suggesting altered auditory processing and sensorimotor gating.

Results: Whole-cell patch-clamp recordings from the calyx of Held-MNTB synapses demonstrated enhanced excitatory postsynaptic currents (EPSCs), increased release probability and reduced readily releasable pool (RRP) size in Cb1r knockout mice. We observed comparable results with pharmacological CB1R blockade with AM251 (CB1R antagonist) in control littermate acute brain slices, indicating the functional CB1R expression at the calyx terminal.

Conclusions: These findings suggest that loss of CB1R signaling alter glutamatergic synaptic transmission and plasticity in the auditory brainstem, leading to impaired auditory processing.

Ongoing studies aim to dissect presynaptic versus postsynaptic contributions of ECS dysfunction to ASD-related auditory phenotypes.

Special Symposium - COMMeND

3:00 p.m. - 5:00 p.m.

Meeting Room 208ABC, Floor 2

Young Investigators Symposium From the Commend Otitis Media Researcher Network

Regie Lyn Santos-Cortez, *University of Colorado Anschutz Medical Campus*

Young Investigators Symposium From the Commend Otitis Media Researcher Network

Diego Preciado¹

¹*Children's National Hospital*

Diego Preciado, *Children's National Hospital*

Individual Abstract: N/A

Young Investigators Symposium From the Commend Otitis Media Researcher Network

Allen F. Ryan¹

¹*University of California, San Diego*

Allen F. Ryan, *University of California, San Diego*

Individual Abstract: N/A

Results From the First Cohort of COMMeND, an R25-Funded Mentoring Network Program Focused on Scientific Otitis Media Training

Sonja Ziniel¹, Landen Mayher², Diego Preciado³, Allen Ryan⁴, Rita Lee², Regie Lyn Santos-Cortez²

¹*University of Colorado Anschutz Medical Campus, Children's Hospital*, ²*University of Colorado Anschutz Medical Campus*, ³*Children's National Hospital, George Washington University School of Medicine*, ⁴*School of Medicine, University of California San Diego*

Sonja Ziniel, *University of Colorado Anschutz Medical Campus, Children's Hospital*

Individual Abstract: Background: To ensure a steady pipeline of Otitis Media (OM) researchers we designed a global mentoring network program that focused on (1) mentoring relationships between early-stage investigators and established faculty, (2) a curriculum of activities for scientific and non-scientific training, and (3) awards for conferences and skill-building short courses. Our first cohort of 14 mentees and 18 mentors started in June 2024.

Methods: We describe the activities of the first year of the mentoring network and report the 14 mentees' expectations, experiences, satisfaction, and accomplishments as measured through pre-, interim- and post-surveys as well as feedback after the webinars.

Results: 57% of mentees were female and identified with underrepresented minorities. Eleven mentees are US-based while 3 are international. Three have positions as junior faculty, 2 are residents, 4 postdocs, 4 predocs, and one is a medical student. Nine mentees selected one mentor (64%) and 5 mentees selected 2 mentors (36%). Most mentees reported in the end-of-year survey to have met with their mentor(s) 2-3x since the start of the academic year. Nearly all mentees reported that the mentoring sessions were either mostly (31%) or very (62%) productive, they felt encouraged and supported by their mentor(s) at every (86%) or most (14%) meetings, and that the meeting frequency was adequate (86%). All mentees felt their mentors were a good fit (100%) and 93% reported that the relationship with their mentor either mostly (50%) or completely (43%) met their expectations, with several mentees expecting the relationship to continue after the end of their tenure as mentee. Most mentees (71%) were very satisfied with their growth throughout the mentorship program, with the remainder being mostly satisfied (29%). The latter mentees elaborated that they wished they had fewer conflicting demands on their time so they could have taken more advantage of the program's offerings. Nevertheless, all mentees (100%) reported that the mentorship program completely met their original needs when they joined the program and all (100%) were very satisfied with the mentorship program overall.

Conclusions: The first cohort of the mentoring network program reported extremely positive experiences. Mentees aim to keep in contact with their new peer network and mentors and can show personal growth and research accomplishments in the field of OM, not possible without this mentoring network structure. A well-designed and funded mentoring network program, such as COMMeND, could be the key to replenishing the numbers of OM researchers.

A Novel Self-Assembling Ion Gel for the Noninvasive Treatment of Acute Otitis Media

Ananya Sahu¹, Colin Houts¹, Alysha Hunter², Eden Tanner², Michelle Calabrese¹

¹*University of Minnesota*, ²*University of Mississippi*

Ananya Sahu, *University of Minnesota*

Individual Abstract: Background: Due to the selective permeability of the tympanic membrane, there is currently no FDA-approved, local, noninvasive treatment for acute otitis media (OM). Here, we explore a novel class of chemical permeation enhancers—choline-based ionic liquids (CBILs)—to enhance the permeation of drugs across the tympanic membrane.

Methods: CBILs have proven to be highly potent and safe permeation enhancers in transdermal delivery applications due to their constituent cations and anions, which act synergistically to traverse impermeable lipid bilayers. To treat acute OM locally, CBILs can be further combined

with poloxamer 407 (P407), an FDA-approved polymer widely used for its thermoresponsive solution-to-gel self-assembly behavior. Thus, an ion gel formulation consisting of P407, CBILs, and drug can be designed to flow into the ear canal as a liquid at room temperature and stiffen upon contact with the warm eardrum to allow sustained drug delivery.

Results: We report, for the first time, that P407 undergoes micelle formation, thermoresponsive transition, and gelation in the presence of CBILs. However, results from rheology and small-angle x-ray scattering suggest that P407 self-assembly mechanisms in aqueous CBIL solvents differ from those in water. First, micelles are present at ambient temperatures, indicating an increased propensity for micelle formation in these CBIL solvents. Second, despite the favorable existence of micelles, much larger concentrations of P407 are required to induce the thermal transition to form a gel. Lastly, P407 micelles are significantly smaller and assemble into a different cubic phase compared to those in water.

Conclusions: From these results, we hypothesize that CBILs fundamentally change the structure and assembly of P407 micelles. By uncovering mechanistic knowledge on P407 self-assembly in these novel aqueous CBIL solvents, an ion gel can be developed towards a noninvasive treatment for acute OM, precluding the need for high-dose antibiotics and invasive interventions.

Crosstalk Between Pathways for Antibiotic Tolerance and Horizontal Gene Transfer in an Otitis Media Pathogen

Mary Marino¹, Evangeline Williams¹, Karan Bamb¹, Regan Schroeder¹, Kevin Lu¹, Katie Quan¹, Azad Ahmed², Garth Ehrlich¹, Joshua Chang Mell¹

¹*Drexel University College of Medicine*, ²*Center for Genomic Sciences, Institute for Molecular Medicine and Infectious Disease*

Mary Marino, *Drexel University College of Medicine*

Individual Abstract: Background: Bacterial middle ear infections often persist when antibiotic treatments fail because of genetic resistance or physiological tolerance. *Haemophilus influenzae* is the most prevalent bacterial pathogen in otitis media and is well-known for its natural competence, a potent mechanism of horizontal gene transfer that spreads antibiotic resistance genes among strains. *H. influenzae* also forms biofilms, which contain metabolically dormant cells that tolerate antibiotics, thereby contributing to persistent infection risk. Nutrient starvation triggers dormancy via the well-conserved ‘stringent response’ by production of the second messenger guanosine tetraphosphate, which was previously shown to induce dormancy and antibiotic tolerance in other bacteria. We hypothesized that the stringent response regulates natural competence via an effect on purine pools and contributes to the spread of resistance genes.

Methods: To determine whether the stringent response plays a role in natural competence, we produced stringent response mutants ($\Delta relA$ and $\Delta relA \Delta spoT$) and characterized their phenotypes. Knockouts in the laboratory strain Rd were produced using an overlap PCR method and natural transformation. Both mutants were confirmed by PCR and whole-genome sequencing. Growth defects in planktonic culture were measured using a shaking-incubating plate reader. For 24-hour static culture biofilms, we measured: (a) biofilm biomass by crystal violet; (b) metabolic activity by a luminescence-based ATP assay; (c) viability and antibiotic

tolerance using plating-based assays. Natural competence phenotypes were measured by culturing cells in competence-inducing media and measuring natural transformation of antibiotic resistances from donor DNA into recipient cells by a plating-based assay. Finally, to distinguish among possible mechanisms for the stringent response's effect on competence gene expression, we performed transcript profiling by RNA-seq.

Results: Stringent response mutants showed: (a) slight changes in planktonic growth; (b) strong defects in biofilm-induced antibiotic tolerance; (c) strong defects in natural competence. Notably, the $\Delta relA \Delta spoT$ double mutant showed unexpected distinct phenotypes from the $\Delta relA$ single mutant, including strong viability defects in competence-inducing conditions. RNA-seq results excluded our initial model of decreased purine repression but instead found that a subset of competence genes has delayed expression, particularly for genes at the ends of long competence-induced operons.

Conclusions: Our data show that the stringent response contributes to natural competence, possibly due to a direct role for guanosine tetraphosphate in supporting transcriptional elongation through competence genes. The effect of the stringent response on natural competence implies that targeting the stringent response would not only potentiate antibiotic effectiveness but also block the spread of antibiotic resistance genes.

Condition-Specific Roles of the Virulence Factor Msf in Regulating Adhesins, Stress Responses, and Biofilm Persistence in *Haemophilus Influenzae*

Evangeline Williams¹, Mary Marino¹, Jocelyn Hammond¹, Laura Anastor-Walters¹, Bhaswati Sen², Azad Ahmad¹, Steven Lang², Sergey Balashov², Benjamin Janto¹, Garth Ehrlich¹, Joshua Chang Mell¹

¹*Drexel University College of Medicine*, ²*Center for Genomic Sciences, Institute for Molecular Medicine and Infectious Disease, Drexel University College of Medicine*

Evangeline Williams, *Drexel University College of Medicine*

Individual Abstract: Background: Nontypeable *Haemophilus influenzae* (NTHi) is a human-restricted bacterium that colonizes the nasopharynx of children and is a leading cause of otitis media (OM). While most acute infections resolve spontaneously or with antibiotics, 12–26% progress to chronic or recurrent OM, a condition that drives substantial healthcare costs and may cause delayed language development or hearing loss. NTHi persistence is strongly associated with biofilm formation, which protects cells from immune clearance. NTHi requires cellular adhesins for early attachment within the nasopharynx and adhesins also promote self-aggregation and biofilm growth.

Methods: We investigated the role of the virulence factor *msf*, previously implicated in macrophage survival and chinchilla OM persistence, under diverse growth conditions. A yeast two-hybrid (Y2H) screen was performed using NTHi prey libraries and *msf* as bait to identify candidate protein–protein interactions. Global transcriptional responses were profiled by RNA sequencing in shaking cultures (exponential and stationary phase) and in 24-hour static cultures, separate supernatant and well-associated biofilm fractions. Differentially expressed genes (DEGs) and pathways were compared across strains and conditions.

Results: Transcriptomic analyses revealed that msf deletion exerted highly condition-dependent effects on gene expression. In shaking cultures, the Δ msf mutant showed limited transcriptional changes, with significant downregulation of only four genes, including two encoding surface adhesins (Hia and a YadA ortholog). In contrast, in 24-hour static cultures, msf deletion produced hundreds of DEGs. Notably, in well-associated biofilm fractions, Δ msf disrupted key physiological pathways, including broad downregulation of translation machinery and concurrent upregulation of protein turnover processes. These dysregulations were specific to the well-associated fraction and absent in supernatant populations, suggesting a role for msf in adapting to the nutrient- and oxygen-limited biofilm niche.

Conclusions: Our findings demonstrate that the msf accessory gene facilitates regulation of outer-membrane protein trafficking, environmental sensing and nutrient adaptation in NTHi. Our Y2H results indicate a likely interaction with an autotransporter adhesin with conserved coiled domain motifs. By modulating stress responses pathways, msf enhances persistence within host-relevant conditions. These results position msf as a central contributor to biofilm physiology and chronic infection and highlight it as a promising therapeutic target to disrupt NTHi biofilm-associated disease.

Non-Typeable Haemophilus Influenzae Small RNAs in the Middle Ear During Otitis Media

Kylie Krohmaly¹, Talulah Lametrie², Hengameh Behzadpour¹, Diego Preciado¹

¹Children's National Hospital, ²George Washington University

Kylie Krohmaly, *Children's National Hospital*

Individual Abstract: Background: Otitis media (OM) is a prevalent condition causing many pediatric medical visits, with a global incidence rate of 10.85%. Non-typeable Haemophilus influenzae (NTHi) is the most common pathogen in acute OM and has been associated with middle ear mucous production. Bacterial small RNAs (sRNAs) are 50-500 nucleotide sequences that function like mammalian microRNAs, using sequence complementarity to bind a transcript target and regulate gene expression. We postulate that bacterial sRNAs are involved in cross-kingdom RNA interactions (CRIs) and modulate human mRNA function in OM. Our aim was to identify NTHi sRNAs present in human middle ear effusion samples (MEEs) during OM, and bioinformatically predict which human transcript targets they may influence.

Methods: We collected MEEs from 6 individuals that were undergoing OM exacerbation and tympanostomy tube drainage, then extracted and sequenced total sRNA (Illumina). Using RNACentral and Bacterial small RNA Database, we gathered 3,301 NTHi-specific sRNA sequences to create a sequence alignment index (kallisto) to identify NTHi sRNAs present in these samples. We calculated the hybridization energy (IntaRNA) between 163 sRNAs that were present in at least one sample and over 98,000 3' untranslated regions (UTRs) from over 22,000 human genes to identify any potential human transcript targets of the sRNAs. Finally, we used MetaScape to identify the top 20 pathways predicted to be enriched within the top 250 targets of 8 sRNAs present in the majority of samples.

Results: We identified 375 NTHi sRNAs present in at least one sample, and 25 NTHi sRNAs present in the majority of samples. Total number of interactions for each sRNA varied between 619 – 5338 3'UTRs from 303 – 2330 unique genes. The lowest energy (designates most stable

interactions) for each sRNA was between 25.79 – 45.97 kcal/mol. While the most common enriched pathways for the 8 sRNAs included cell membrane organization and transport, neural and muscle development, and various forms of metabolism, we report 5 sRNAs with immune-related processes (4 sRNAs), Wnt signaling (3), and glycoprotein metabolism (2) as enriched pathways.

Conclusions: Future research, such as a larger sample size and in vitro confirmation of the identified CRIs is needed. However, this study highlights a potential role for NTHi sRNAs to participate in CRIs in OM.

A Human Middle-Ear-On-A-Chip Platform for Modeling Otitis Media

Arwa Kurabi¹, Henry Hwang¹, Chanond Nasamran¹, Kwang Pak¹, Shaochen Chen¹, Rick Friedman¹, Allen Ryan¹

¹*University of California, San Diego*

Arwa Kurabi, *University of California, San Diego*

Individual Abstract: Background: Three-dimensional (3D) culture systems have enabled development of human tissue models that recapitulate organ-specific architecture and cellular complexity. Otitis media (OM) is one of the most common childhood illnesses, yet study of human ME gene expression has largely been restricted to late-stage disease. To address this gap, we developed a middle-ear-on-a-chip (MEoC), a microfluidic platform to model the early events in OM using primary human ME mucosal tissue.

Methods: The MEoC consists of a two-chamber microfluidic system separated by a semi-permeable membrane. Fresh human ME mucosal explants were placed in the epithelial (ME) chamber on a semi-permeable membrane, while human endothelial cells were cultured in the vascular chamber side of the membrane. This system allows for the addition of pathogenic bacteria to the ME chamber and perfusion of human leukocytes under dynamic flow through the vascular chamber. To define transcriptional programs within the device, the human ME mucosa was profiled by single-cell RNA sequencing (Chromium 10x Genomics) under baseline conditions and after 6 hrs of nontypeable *Haemophilus influenzae* (NTHi) infection, complemented by qPCR validation of selected genes and histological analysis of tissue structure. Clusters were identified by principal component analysis (PCA) and annotated using canonical marker genes.

Results: The MEoC preserved key features of the native human ME mucosa and enabled infection-driven remodeling of epithelial and stromal compartments. scRNA-seq of device-cultured tissue revealed epithelial and stromal cell clusters at baseline, with NTHi exposure inducing inflammatory fibroblast expansion and stress-response programs. MUC1 was constitutively expressed, suggesting barrier maintenance, while MUC4 was reduced after infection, indicating impaired epithelial restitution. COL1A1 expression persisted across conditions, whereas COL3A1 was specifically induced by NTHi, mirroring fibroinflammatory activation as has been seen in airway-on-chip models.

Conclusions: The MEoC offers a scalable and translational platform for studying the human ME microenvironment. By integrating scRNA-seq at baseline and infected tissue within the device, this system provides a human-relevant tool for identifying mechanisms of OM pathogenesis and

therapeutic targets, screening antimicrobial strategies, and studying ME epithelial–immune cross-talk.

Impact of Triplication of Ifnrs on Otitis Media and Hearing Loss in a Mouse Model of Down Syndrome

Regina Gendzelevski Kelmann¹, Cong Tian¹, Kelly Sullivan², Joaquin Espinosa², Peter Steyger¹

¹*Creighton University*, ²*University of Colorado Anschutz Medical Campus*

Regina Gendzelevski Kelmann, *Creighton University*

Individual Abstract: Background: Otitis media is the most common cause of hearing loss in children with Down syndrome (DS), affecting as many as 67% of children within their first 12 months, and persisting in 60% of children with DS at 6–7 years old. Hearing loss during early childhood can significantly exacerbate pre-existing cognitive and speech delays present in children with DS, leading to greater delays in educational outcomes and social communication. The Dp(16)1Yey (Dp16) mouse model which mimics human DS phenotypes, including otitis media, craniofacial abnormalities and hearing loss. Triplication of genes for interferon receptors (IFNRs) have been linked to craniofacial anomalies in Dp16 mice and normalization of IFNR gene dosage in Dp16 mice significantly reduced the degree of craniofacial anomalies. Therefore, we investigated whether normalization of IFNR gene dosage attenuated OM and hearing loss in Dp16 mice.

Methods: Auditory brainstem response (ABR) thresholds and distortion product otoacoustic emissions (DPOAEs) were collected with a Tucker-Davis Technologies RZ6 system from Dp16 mice, wild-type (WT) littermates, and Dp16 mice with normalized IFNR gene dose (Dp162xIFNRs). Sections of fixed temporal bullae were assessed for presence of otitis media

Results: ABR thresholds were significantly elevated in Dp162xIFNRs (n=4) and Dp16 (n=4) mice compared to WT. No statistical differences in ABRs were observed between Dp162xIFNR and Dp16 mice. The incidence of otitis media was significantly higher in Dp162xIFNR (n=4) and Dp16 (n=4) mice compared to WT, however, no differences were observed between Dp162xIFNR and Dp16 mice (N=10).

Conclusions: Normalized IFNR gene dosage mice did not attenuate the degree of OM or hearing loss present in Dp16 mice (with IFNR gene triplication). This highlights the genetic and physiological complexity of OM and hearing loss in Dp16 mice. Characterizing the structural and functional properties of the middle ear in Dp16 mice will enable development of interventions to reduce hearing loss in this population.

Multi-Omic Studies on Cholesteatoma, Eardrum and Middle Ear Mucosa

Antonio Bon-Nieves¹, Eric Larson², Arwa Kurabi³, Regie Lyn Santos-Cortez⁴

¹*University of Connecticut*, ²*University of Pennsylvania*, ³*University of California San Diego*,

⁴*University of Colorado Anschutz Medical Campus*

Antonio Bon-Nieves, *University of Connecticut*

Individual Abstract: Background: Secondary acquired cholesteatomas (SACH) are histologically benign but locally destructive lesions characterized by the abnormal accumulation of keratinizing squamous epithelium and keratin debris within the middle ear or mastoid. Several mechanisms have been proposed for SACH formation, including epithelial migration, squamous metaplasia, and basal cell hyperplasia. Increasingly, SACH is also viewed through the lens of a chronic wound-healing hypothesis, whereby persistent cycles of injury, inflammation, and fibrosis promote maladaptive epithelial growth and expansion. In this study, we profiled middle ear samples using single-cell RNA sequencing (scRNA-seq) and contextualized our findings within the molecular basis of SACH, incorporating genomic, transcriptomic, proteomic, metabolomic, microbiome, and computational modeling studies.

Methods: With ethical approval, intraoperative samples of human cholesteatoma, granulation tissue, normal-appearing middle ear mucosa, and tympanic membrane (TM) were collected from consenting patients. Tissues were processed and submitted for scRNA-seq. For the literature review, a comprehensive PubMed search was performed using terms related to cholesteatoma pathogenesis, including “genome,” “transcriptome,” “array,” “microbiome,” “proteomics,” “exome,” and “RNA sequencing.” Three reviewers (RSC, AK, and ABN) independently screened studies for inclusion.

Results: Preliminary scRNA-seq analysis identified four distinct populations in cholesteatoma, keratinocytes (KRT14⁺), lymphocytes (PTPRC⁺), ciliated epithelial cells (HYDIN⁺), and stromal/fibroblast (COL1A1⁺ and COL3A1⁺). For the review, 108 studies met the initial screening criteria after duplicate removal. Integrating our scRNA-seq findings with multi-omic literature suggests that current models of cholesteatoma formation are incomplete and that advanced molecular tools can refine our understanding of disease mechanisms.

Conclusions: This study provides a comprehensive multi-omic perspective on SACH, supporting the concept that cholesteatoma behaves as a dysregulated wound-healing disorder. Persistent stromal activation (COL1A1/COL3A1⁺ fibroblasts), aberrant immune–epithelial crosstalk, and enrichment of signaling pathways such as TGFβ, periostin, and HSPG highlight molecular mechanisms that perpetuate chronic injury and epithelial expansion. Together, these findings suggest that cholesteatoma progression represents a failed transition from acute, protective tissue repair to chronic, fibrotic remodeling. This framework highlights candidate therapeutic pathways that could be targeted to develop medical treatments beyond the current surgical gold standard.

Integrating Leadership Development Into Commend

Rita Lee¹, Sonja Ziniel², Landen Mayher², Diego Preciado³, Allen Ryan⁴, Regie Lyn Santos-Cortez²

¹*University of Colorado School of Medicine*, ²*School of Medicine, University of Colorado Anschutz Medical Campus*, ³*Children's National Hospital*, ⁴*University of California, San Diego*

Rita Lee, *University of Colorado School of Medicine*

Individual Abstract: Background: Effective leadership is critically important to foster high-performing teams, to improve problem-solving skills and to reduce burnout. Many research-

oriented mentorship programs focus on grant writing and research skills, with less emphasis on leadership skills such as communication, feedback, or collaboration. We sought to integrate leadership development training into our COMMeND mentoring network program.

Methods: The leadership curriculum was developed using existing leadership curricula as a framework. Leveraging adult learning theory, sessions were interactive and directly related to the work environment of mentees and mentors. We describe the leadership curriculum of the first year of the mentoring network.

Results: Our first cohort of 14 mentees and 18 mentors started in June 2024. In the first year, the leadership development curriculum included effective communication, feedback skills, collaborative leadership, resilience, and effective teaching strategies. Each session was 40 minutes to one hour in duration and included reflective activities. When asked whether how likely they were to apply the information learned, 100% responded positively (“pretty likely” or “very likely”) for Effective Communication and Responsible Conduct of Research sessions, 91% for Collaborative Leadership, 83% for Grant Writing, and 75% for Implicit Bias. Participants expressed that the sessions provided broad insights and perspectives that are added value to research-oriented content: “the webinars have been great and gotten me to think from different angles.” “I gained insights into effective communication, leveraging individual strengths, and navigating complex professional environments with a solutions-focused mindset. As well, I have deepened my understanding of creating and sustaining an inclusive environment. This experience has equipped me with actionable strategies to foster respect and inclusivity within teams, enhancing collaboration and morale.”

Conclusions: Inclusion of leadership development into a mentoring network is feasible and well received. Fostering leadership skills may improve team inclusivity, functioning, and productivity. Long-term impact and outcome data should be measured.

Mini Podium 5: Hearing and Balance in the Balance: Injury of Inner Ear Neurons and Synapses

4:15 p.m. - 5:15 p.m.

Meeting Room 209ABC, Floor 2

Moderators: Afagh Farhadi and Mark Rutherford

Combined Effects of Mild Traumatic Brain Injury and Noise Exposure on Auditory and Vestibular Function

4:15 p.m. - 4:30 p.m.

Federica M. Raciti^{*1}, Nadine Kerr², Suhrud Rajguru³

¹*University of Miami*, ²*Miami Project to cure paralysis, University of Miami*, ³*University of Miami, Bruce W. Carter Department of Veterans Affairs Medical Center*

Background: Traumatic brain injury (TBI) is a complex public health challenge whose consequences extend well beyond cognitive and motor dysfunction. Among the less recognized yet clinically significant outcomes are sensorineural deficits of the auditory and vestibular

systems, which can manifest as hearing loss, imbalance, and spatial disorientation. Damage to these sensory structures and their associated neural pathways impairs communication and orientation, reducing functional independence and overall quality of life. Individuals at risk for mild TBI (mTBI), including military personnel, construction workers, and athletes, are also often exposed to moderate-to-high levels of environmental noise, a well-established cause of noise-induced hearing and vestibular loss. The common scenario in which mTBI is followed by intense noise raises critical questions about whether a prior head injury increases vulnerability to later acoustic trauma or alters the trajectory of sensory and cognitive recovery.

Methods: We investigated short- and long-term changes in auditory, vestibular, and cognitive function in ecologically valid rodent models of mTBI combined with either high- or low-intensity broadband noise exposure. Closed-head injuries were induced using an accelerated weight-drop system (450 g from 1 m). Four days later, an interval associated with recovery of gross motor and cognitive performance, animals were exposed to either high-intensity noise (4–16 kHz, 110 dB SPL, 1 hr) or low-intensity noise (4–16 kHz, 95 dB SPL, 1 hr). Corresponding noise-only cohorts were evaluated in parallel. Functional outcomes were assessed using auditory brainstem responses (ABRs), cervical vestibular evoked myogenic potentials (cVEMPs), and a battery of cognitive tests, with measurements obtained from baseline up to 28 days after noise exposure.

Results: Initial analyses show that when high-intensity noise followed mTBI, auditory and vestibular outcomes were indistinguishable from those produced by high-intensity noise alone at both acute and extended time points. In contrast, when low-intensity noise followed mTBI, a history of head trauma unmasked significant compounding effects, with more pronounced and persistent alterations in sensory and cognitive measures than with low-intensity noise alone. These findings suggest a “ceiling effect” limited to peripheral sensory outcomes, in which the permanent damage caused by high-intensity noise masks any additional influence of prior mTBI. Central auditory and vestibular processing has not yet been examined and may reveal further interactions.

Conclusions: These results underscore the need to consider sensory and cognitive outcomes alongside traditional neurological assessments in TBI research and clinical practice. Recognizing how a history of mTBI modulates the impact of different noise exposures will be critical for developing protective strategies and targeted interventions for at-risk populations.

Cochlear Implant Insertion Pressure Transients Induce Vestibular Temporary Threshold Shifts

4:30 p.m. - 4:45 p.m.

Nathaniel Greene*¹, Anna Dondzillo²

¹*Applied Research Associates*, ²*University of Colorado School of Medicine*

Background: Patients undergoing cochlear implantation may experience loss of (generally low frequency) residual hearing, but frequently (> 1/3 of patients) exhibit a transient balance dysfunction following surgery. We have previously reported that the cochlear implant (CI) insertion generates a high intensity pressure transients in the inner ear. . These transients are generated by all CI types and manufacturers, are less frequent but equal in intensity during slow or automated insertion, and appear to result from contact between the tip of the electrode array

and the walls of the cochlea. Furthermore, these transients are observed in both the cochlea, and throughout vestibular labyrinth. These transients show high peak pressures, which can exceed 170 dB SPL peak (equivalent pressure in the ear canal), but long in duration, with A-durations over 100 ms, thus low frequency (peak energy of 5-10 Hz).

The cochlea is not sensitive to these low frequencies, and indeed patients undergoing CI implantation under local anesthesia do not report hearing noise during the insertion. However, these frequencies are within the range of head motions to which the vestibular system is sensitive. We therefore hypothesize that this low frequency impulse noise exposure contributes to vestibular injury and balance dysfunction after CI implantation.

To investigate the underlying mechanisms of injury from the CI surgical insertions, we developed a novel translational noise exposure model in the Mongolian Gerbil (*Meriones unguiculatus*).

Methods: Intracochlear pressure transients previously recorded in human cadaveric specimens were transformed by the gerbil middle ear to generate a vibratory stimulus that, when delivered by an electrodynamic shaker (Brüel and Kjær), generated an equivalent exposure in the gerbil inner ear. Auditory brainstem responses (ABR) and vestibular sensory evoked potentials (VSEPs) were assessed in young adult gerbils before and at several time points after exposure to 1000 repetitions of this impulse noise exposure. The immunofluorescent anti-CtBP2 (ribbon synapse marker), anti-GluR2 (afferent glutamate receptors) and myosin7a (a hair cell marker) were then examined in the cochlea and vestibular organs to identify loss of sensory hair cells and synapses.

Results: Exposed gerbils showed a transient decrease in amplitudes and increase in thresholds in VSEP but not ABR amplitudes 1-2 weeks after noise exposure, which generally recovered by week 3. Physiological estimates of hearing and balance function are compared to loss of sensory cells and afferent nerve terminals via immunohistochemical assessments of the cochlea and vestibular organs in each animal.

Conclusions: Results suggest that intracochlear pressure transients from cochlear implant insertion may provide a substantial contribution to the balance dysfunction resulting from cochlear implantation, and highlights the importance of soft surgical techniques and otoprotectants.

Funding: NIH/NIDCD: R21-DC017809, AHRF Discovery Grant

Tyrosine Kinase Inhibition Restores Inner Hair Cell Synapses After Kainate-Induced Excitotoxicity

4:45 p.m. - 5:00 p.m.

Anissa Rym Saidia¹, Tong Yang^{*1}, Ichiro Furuta¹, Florence François¹, Jean-Luc Puel¹, Jing Wang¹

¹*Institute for Neurosciences of Montpellier (INM), University of Montpellier, INSERM*

Background: Cochlear synaptopathy is recognized as an early driver of auditory dysfunction. Type I spiral ganglion neurons (SGNs) connect with inner hair cells (IHCs) to convey sound information to the brainstem. Disruption of this synaptic interface is an early hallmark of noise

exposure, ototoxic drug treatment, and cochlear aging. Excitotoxicity, triggered by excessive glutamate release, is thought to initiate this degenerative process, leading to ribbon synapse loss and reduced auditory nerve output despite preserved hearing thresholds and outer hair cell (OHC) function, a condition underlying auditory synaptopathy and “hidden hearing loss.” Macrophages, which express tyrosine kinase receptors, are strongly activated after cochlear injury and contribute to this pathology. Here, we investigated whether M1, a selective tyrosine kinase inhibitor, can protect and restore IHC-SGN synapses following excitotoxic damage.

Methods: We first used neonatal (P3) mouse cochlear explants to assess the protective effect of tyrosine kinase inhibition on kainate-induced synaptic loss in vitro. To explore underlying mechanisms, we analyzed molecular markers of synaptic rescue. We then established an in vivo model of excitotoxic cochlear synaptopathy based on an acute semicircular canal injection of kainate in adult CBA/J mice and evaluated the therapeutic efficacy of transtympanic M1 delivery.

Results: Tyrosine kinase inhibitor M1 treatment significantly preserved ribbon synapse in kainate-exposed explants. In vivo, kainate injection reduced the wave I amplitude of the auditory brainstem response (ABR) and the number of ribbon synapses, while distortion product otoacoustic emissions (DPOAEs) and hearing thresholds remained intact, consistent with auditory synaptopathy. Transtympanic M1 administration significantly promoted the recovery of both ABR wave I amplitude and synapse numbers compared with controls.

Conclusions: Tyrosine kinase inhibition protects synaptic ribbons after excitotoxic injury in both in vitro and in vivo models. These findings identify tyrosine kinase inhibition as a promising therapeutic strategy for cochlear synaptopathy and hidden hearing loss.

Molecularly Defined Spiral Ganglion Neuron Subtypes Have Different Vulnerabilities to Noise-Induced and Age-Related Synaptopathy in Mice

5:00 p.m. - 5:15 p.m.

Joy Franco^{*1}, Taylor Copeland¹, Ryan Merrow¹, Lisa Goodrich¹

¹*Harvard University*

Background: Noise-induced and age-related cochlear synaptopathy impacts a subset of Type I spiral ganglion neurons (SGNs), but a causative link between identity and vulnerability has yet to be established. Type I SGNs are molecularly defined as Ia, Ib, or Ic, with Ia's (likely corresponding to high-spontaneous firing rate, SR) primarily innervating the pillar and Ic's (low-SR) the modiolar side of inner hair cells (IHCs). Fewer low-SR SGNs are detected in recordings from noise exposed cats and guinea pigs, and in old gerbils, suggesting Ic's are selectively vulnerable. However, this trend was not observed in mice. Innervation patterns along the pillar-modiolar axis are not strict and therefore cannot serve as a definitive proxy for molecular subtype. Thus, we addressed this knowledge gap directly using genetic labelling and by manipulating SGN identity.

Methods: We assessed molecular subtype-specific synaptic vulnerability using reporter mice to drive cytosolic expression of tdTomato in genetically-defined SGNs. Ntng1Cre;Ai14 mice were used to distinguish Ib and Ic's (tdTomato⁺) from Ia's (tdTomato⁻) for aging and noise exposure

experiments. For further analysis of noise-induced synaptopathy, Calb2CreERT2;Ai14 and Lypd1CreERT2;Ai14 mice were used to label Ia and Ic's, respectively.

To directly assess the impact of molecular identity on synaptic vulnerability to noise exposure, we leveraged Runx1 conditional knock-out (CKO) mice in which Ib and Ic's take on a Ia-like identity, reflected both in the morphology of their synapses and their auditory brainstem responses.

We quantified synaptic properties in cochlear tissue immunolabelled to detect CALB2 (IHCs), CtBP2 (Ribeye+ ribbons), Homer1 (postsynaptic densities, PSDs), and, where applicable, for tdTomato (fluorophore signal amplification). CtBP2 and Homer1 puncta were reconstructed in Imaris and analyzed with a custom, user-informed Python/ImageJ pipeline for quantification of synaptic density and identity, orphan number, and the volumes of the pre- and postsynaptic puncta associated with Ia, Ib, and Ic terminals.

Results: Ia synapses showed a striking resilience, with most Ia synapses surviving despite overall decreases in synaptic density. Mean Ia PSD volumes were significantly larger than those of Ib/Ic's. While Ia PSD volumes increased with age, those of Ib/Ic's were unchanged. Conversely, Ia PSDs volumes were unaffected by noise exposure, but the average Ib/Ic volume decreased.

Runx1 CKO mice retained significantly more synapses after noise exposure. This was reflected both in the overall number of synapses and in the significant reduction in the number of orphaned Homer1 puncta, which appeared after noise exposure in littermate controls but not in Runx1 CKO mice.

Conclusions: Our genetic labelling and reprogramming experiments establish a causal relationship between Ia identity and synaptic resilience. In tracking PSD volume and identity, we find compelling evidence that this resilience may encompass an ability to repair or remodel post-synaptic structures. These findings hold importance for gene-therapy research within and beyond the auditory system.

Mini Podium 6: Binaural Hearing: From Neural Responses to Human Psychophysics

4:15 p.m. - 5:15 p.m.

Ballroom B, Floor 3

Moderators: Chris Stecker and Rachel Cassidy

Dendrite-Based Delay Lines Shape the Computation of Interaural Time Differences in Neurons of the Medial Superior Olive

4:15 p.m. - 4:30 p.m.

Jared Casarez¹, Rebecca Voglewede¹, Bradley Winters¹, Ken Ledford¹, Nace Golding*¹

¹*University of Texas at Austin*

Background: In mammals, neurons of the medial superior olive (MSO) encode interaural time differences (ITDs) of sounds originating from different horizontal locations. To accomplish this task, MSO neurons segregate ipsilateral and contralateral excitatory inputs onto lateral and medial dendritic branches and signal their temporal coincidence through variations in firing rate. This spatial tuning requires a cell or circuit-based delay to offset acoustic disparities. The source of this “internal delay”, alternately assumed to arise from axonal delay lines or postsynaptic inhibition, remains controversial. Here we present anatomical and physiological evidence that the dendrites themselves are a major source of internal delay.

Methods: We used 2-photon fluorescence-guided patch recordings to measure propagation delays from distal dendrites of MSO neurons to the soma (gerbils aged 17-40; recording range up to 150 μm from the soma). Compartmental modeling was implemented in NEURON using reconstructions of 40 fully intact biocytin-labeled MSO neurons from Bondy et al., 2020. Channel models were implemented as in Matthews et al., 2010 and Khurana et al., 2011. Synaptic conductances were modeled as alpha functions ($\tau=0.27$ ms), and either activated individually at dendritic locations or in dendro-somatic sequences (1 m/s; (Callan et al., 2023)). All synapses exhibited a release probability of 0.45 (Couchman et al., 2011).

Results: In paired dendritic and somatic recordings we observed that delays of simulated EPSPs propagating from distal dendritic regions to the soma were diverse and correlated with local dendritic morphology and membrane time constant, the latter of which varied between 170 and 730 μs . Morphological analyses of reconstructed MSO neurons revealed striking variations in the degree of asymmetry between medial and lateral dendrites. Our MSO neuron population formed a continuum of medially and laterally biased cells, with up to ~ 2 fold differences between sides in dendritic surface area and length. Accordingly, in NEURON models of MSO neurons, these morphological asymmetries translated into branch-specific distortions in the rise time and duration of EPSPs after propagation to the soma. Across different dendritic locations, rise times of propagated EPSPs at the soma could vary by more than 300 μs . Finally, in simulations of binaural coincidence detection, cells with dendritic asymmetries exhibited differences in best delays (BDs) of up to 150 μs , exceeding the physiological range of interaural time differences (ITDs) reported for the gerbil.

Conclusions: We conclude that the bipolar dendritic arbor of MSO neurons is typically asymmetrical, imposing an asymmetry in the relative timing required for excitatory inputs to drive optimal action potential output. This variation in dendritic morphology exerts a continuum of temporal influences sufficient to shift spatial receptive fields across the full biological range of available ITDs. These shifts likely work together with other mechanisms to enable MSO neurons to stably encode spatial locations along the horizontal plane.

Delay Mismatch Compensation Between Acoustic and Electric Inputs Partially Restores Degraded Interaural Time Difference Coding to Binaural-Bimodal Stimulation

4:30 p.m. - 4:45 p.m.

Maïke Vollmer¹, Merle Berents¹, Andreas Schulz², Frank W. Ohl², Andreas Schulz^{*3}

¹University Hospital Magdeburg, Germany, ²Systems Physiology of Learning, Leibniz Institute for Neurobiology, Magdeburg, Germany, ³Leibniz Institute for Neurobiology

Background: Despite the use of unilateral cochlear implants, directional hearing in subjects with single-sided deafness (SSD-CI users) is typically poorer than that in normal hearing subjects and in subjects using bilateral CIs. Nevertheless, directional hearing in binaural-bimodal (acoustic-electric) stimulation can be improved by compensating the unphysiological delay between acoustic and electric stimulation (Angermeier et al. 2023).

Methods: To identify the deficits of binaural-bimodal processing and to evaluate the effects of delay mismatch compensations on neural ITD sensitivity, we compared ITD tuning metrics and coding efficacy (Fisher information) of single neurons in the inferior colliculus of normal hearing gerbils in response to bimodal stimulation (with and without delay compensations) and to unimodal acoustic and unimodal electric stimulation. To maintain acoustic sensitivity of the implanted ears and, thus, to allow within-neuron comparisons of responses across the three kinds of stimulation, electric stimuli were delivered to the round window. Different strategies were used to perform delay compensations on the single neuron level.

Results: Fisher information was highest in response to unimodal acoustic stimulation, followed by unimodal electric stimulation. In response to bimodal acoustic-electric stimulation, Fisher information was significantly reduced. Artificial compensations of the delay between acoustic and electric inputs shifted the bimodal ITD tuning curves towards the physiological range of ITDs and increased the average Fisher information. Although acoustic and electric stimulus intensities were adjusted in order to obtain similar response strengths to monaural stimulations, maximal response strength to binaural-bimodal stimulation was significantly lower than that to unimodal acoustic stimulation.

Conclusions: Our results support the assumption that artificial delay compensation strategies between acoustic and electric inputs enhance ITD sensitivity to bimodal stimulation and may, thus, improve directional hearing in SSD-CI users. Nevertheless, our data indicate suboptimal neural integration of bimodal stimulation and, to a lesser extent, of unimodal electric stimulation. Additional coding strategies need to be considered to optimize ITD processing in response to bimodal and unimodal electric stimulation.

Supported by DFG VO 640/2-2, Towards Co-Evolution in Human-Technology Interfaces (TACTIC-LIN), and MED-EL

The Influence of Prior Experience on Distance Perception in Fixed and Varying Environments

4:45 p.m. - 5:00 p.m.

Norbert Kopco*¹, Myroslav Fedorenko¹

¹*P.J. Safarik University, Kosice*

Background: Auditory distance perception depends on listeners' ability to adapt to the surrounding acoustics. Prior studies have shown that accuracy improves with implicit calibration in reverberant rooms but not in anechoic conditions, suggesting that "room learning" relies on reverberation-specific cues, though the underlying processes remain unclear. In a previous study [Kopčo and Fedorenko, 2024, ARO PS8 Abstract], we examined how environmental consistency and preceding exposure shape performance, showing that initial exposure to consistent simulated

environments supports accurate distance judgments, evaluated by using the stimulus-response correlation, whereas inconsistent exposure hinders them. In the present work, we extend this analysis by evaluating response biases and by examining the acoustic effects of the environments on the distance cues.

Methods: Three virtual environments were tested (anechoic, classroom center, classroom corner), with sources presented from medial and lateral directions. Stimuli were 5-burst pink noise sequences, level-rovved to minimize loudness cues. On each trial, one of nine distances (15–170 cm, spaced approximately logarithmically) was presented. A run consisted of 45 trials in which the stimulus direction was fixed while the environment could be fixed or randomly chosen on each trial. A session consisted of 8 runs. Participants (N=8) were split into two groups: one began with 6 sessions in a fixed environment followed by 6 in mixed environment, while the other experienced the reverse order.

Results: Overall performance, measured by correlation coefficients, revealed a clear effect of exposure order. The group that began in consistent conditions achieved higher accuracy across all rooms, but their performance declined upon switching to variable conditions, with the magnitude of deterioration depending on room type and source direction. In contrast, the group starting with variable conditions performed significantly worse, and subsequent exposure to consistent conditions yielded only modest improvements. Bias analyses showed systematic underestimation of distances in the anechoic room and overestimation in reverberant rooms, consistent with the use of a single Direct-to-Reverberant Ratio (DRR) mapping across contexts. Performance was generally higher in reverberant environments than in anechoic, and for lateral sources compared to medial ones, indicating effective utilization of both DRR and Interaural Level Difference (ILD) cues when available. The acoustic analysis showed that the cues were considerably altered in the corner vs center of the room.

Conclusions: These findings demonstrate that listeners rely on prior experience to calibrate distance perception in specific environments. This room-specific tuning is disrupted when acoustic conditions vary rapidly, leading the auditory system to collapse multiple contexts into a single generalized mapping. Thus, auditory distance perception is a dynamic, experience-driven process, constantly tuned by the context of the preceding experience.

Timing Matters! –The Benefit of Temporal Fine Structure for Spatial Hearing With Bilateral Cochlear Implants

5:00 p.m. - 5:15 p.m.

Nicole Rosskoth-Kuhl^{*1}, Aurelia Daxer², Konstantin Wiebe³, Jan W. Schnupp⁴, Susan Arndt⁵, Thomas Wesarg³

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Background: The ability to perceive interaural time differences (ITDs) is very important for sound localization and speech understanding in spatially separated speech and noise. Compared

to normal hearing individuals, children and adults with bilateral cochlear implants (biCI) show significantly reduced or even no sensitivity to ITDs. One possible reason for the poorer ITD sensitivity of biCI users, especially with regard to ITDs in the temporal fine structure, might be the lack of bilateral synchronization of the stimulation pulses by the two CIs in the majority of clinical sound processors. An exception to this are the fine structure coding strategies FS4 and FS4p implemented in CI sound processors (SONNET and RONDO) by MED-EL. They encode fine structure information of the low-frequency sound signal components and present it via the four most apical stimulation electrodes in the cochlea. In contrast, CIS-based strategies also implemented in these processors as well as other clinical CI processors do not encode fine structure information.

Methods: In this study, we investigated the ITD sensitivity of biCI children and biCI adults to a low-frequency tone burst (250 Hz) with two different onset and offset ramp durations, 4 ms and 100 ms, using the sound coding strategies FS4 and HDCIS with SONNET 2 sound processors. The audio stimuli were presented bilaterally via the audio inputs of the two processors, and ITD sensitivity was tested using a 2-alternative forced choice lateralization task.

Results: In total, ten adult biCI patients and ten biCI children have been included. For tone bursts with 4 ms ramp duration, containing onset and ongoing ITD information, all biCI patients showed a good and significantly better ITD sensitivity when using FS4 compared to HDCIS, with some patients even showing no sensitivity under HDCIS. The ITD sensitivity for tone bursts with 100 ms ramp duration, i.e., with only ongoing ITD information, was worse compared to 4 ms for both cohorts of biCI users. While around half of the children and almost all of the adults showed ITD sensitivity with FS4 for a ramp duration of 100 ms, this sensitivity was completely lost in all biCI patients when the HDCIS strategy was combined with a 100 ms ramp duration.

Conclusions: In contrast to HDCIS, the fine structure coding strategy FS4 enabled biCI children and biCI adults to achieve significantly better sensitivity to ITDs in low-frequency tones. In addition, biCI patients showed an improvement in their ITD sensitivity when receiving both onset and ongoing ITDs compared to ongoing ITDs only. These findings highlight the importance of fine structure coding strategies for biCI patients to improve their spatial hearing.

Memorial Symposium: Jim Hudspeth

5:15 p.m. - 7:15 p.m.

Ballroom B, Floor 3

Sensing Hair Cells - In Memory of A. James Hudspeth (1945-2025)

Ruth Anne Eatock, *University of Chicago*

Structure and Function of the Mechanotransduction Complex in Hair Cells

David Corey, *Harvard Medical School*

Individual Abstract: Early work on bullfrog saccular hair cells led to the idea that the mechanotransduction channel is directly gated by mechanical stimuli, enabling a very sensitive and very fast response to sound and head movements. Deflection of stereocilia was thought to stretch a hypothetical elastic element—the gating spring—which increases tension on the mechanotransduction channel protein and makes the open state energetically favorable. Stiffness measurements suggested tension of a few piconewtons and a channel gating movement of a few nanometers. Unclear at the time were the identify of the channel and gating spring, the identity of linking proteins that convey tension to the channel, how the channel moves to open, and how force is propagated within the channel protein to promote opening.

Forty years later, some but not all of these enigmas have been solved. Positional cloning of deafness genes has revealed most of the key proteins, including the transduction channels TMC1 and TMC2, subunits LHFPL5 and CIB2, and the tip-link proteins CDH23 and PCDH15. The gating spring is not clearly identified. And how these all work together in a complex remains a mystery.

We and collaborators have suggested that TMC1 shares a structure with the TMEM16 family of ion channels and scramblases, and that it forms a dimeric complex with two independent ion conduction pathways. Cysteine mutagenesis with acute modification during recording confirmed a pore groove bounded by transmembrane domains TM4-7. AlphaFold modeling has hinted at how the channel moves to open, indicating a separation between TM4 and TM6 near the extracellular side. Mutation of residues in these helices change the likelihood that a channel opens. To understand how the tip link is coupled to the channel, we used co-immunoprecipitation with fragments and chimeras of PCDH15 and TMC1. These point to an extracellular domain of PCDH15 binding to an extracellular loop of TMC1. Evolutionary analysis indicated elaboration of the TM1-TM2 loop in the TMC1/2 family, with positive selection of specific residues in mammals. Mutations in this loop also affect gating.

This insight into how sound is converted to a neural signal, now at the level of single atoms, could barely be imagined 40 years ago. Yet questions still remain, and we still view the mechanotransduction complex through a cloudy pane.

On the Critical Thing About Hair-Cell Amplification of Sound-Evoked Vibrations

Pascal Martin, *CNRS / Institut Curie-PSL University / Sorbonne University*

Individual Abstract: The remarkable sensitivity, frequency selectivity, and dynamic range of hearing, together with the widespread observation of otoacoustic emissions, are widely thought to result from an active process. In this talk, I will discuss the possibility that the active process stems from a general principle: operation of the hair cells on the brink of an oscillatory instability, namely a Hopf bifurcation—one example of “critical” phenomena that are widespread in physics. Experiments in an ex vivo preparation of the frog saccule have revealed how spontaneous mechanical oscillations of the hair bundle—the mechanosensory antenna of the hair cell—enhance sensitivity and sharpen frequency selectivity to weak periodic stimuli. Frequency-selective amplification arising from active hair-bundle motility in a hair cell from the frog’s ear is associated with a compressive nonlinearity that shares fundamental features with the

nonlinearity observed in vivo in the mammalian cochlea and, remarkably, in even more evolutionarily distant ears such as the antennal auditory organs of fruit flies and mosquitoes. This specific type of nonlinearity is generic of critical oscillators; its properties do not depend on the candidate mechanism that brings the system on the verge of spontaneous oscillation. Following Jim Hudspeth's vision, I will argue that the necessity of hearing sounds with high sensitivity and frequency selectivity has led evolution to arrive at essentially the same solution—critical oscillators—across diverse auditory systems and species, while allowing for varied implementations of criticality at the molecular, cellular and supra-cellular scales.

Voltage-Gated Ion Channels Shape Hair-Cell Receptor Potentials to Solve Different Problems

Ruth Anne Eatock, *University of Chicago*

Individual Abstract: Hair cells solve different sensory problems by shaping their receptor potentials through specific complements of voltage-gated ion channels. Transduction currents arising in the apical hair bundle initiate receptor potentials that modulate dense arrays of voltage-sensitive ion channels in the hair cell's basolateral membrane. The basolateral channels have different ion selectivities, time courses and voltage ranges, and are present in complements that differ systematically between hair cells of different types. In this way, basolateral membrane properties shape the receptor potential to transmit specific features of the sound or head motion across synaptic contacts to afferent nerve fibers.

Pioneering experiments in Jim Hudspeth's lab alerted us to these possibilities. As a graduate student with Jim in the early 1980s, Rich Lewis performed the first patch clamp experiments on hair cells, revealing high-quality electrical tuning by the basolateral membrane. Through complementary modeling, he showed how the sharp tuning arose from the interplay of voltage-gated calcium current and calcium-gated potassium current. Subsequently, Bill Roberts showed that the calcium and potassium channels cluster near the presynaptic ribbons, with calcium entry regulating both electrical tuning and quantal afferent transmission. These elegant studies on frog saccular hair cells supplied a framework for understanding how basolateral channels shape transmission.

A decade later, we and others began patch clamp studies of type I hair cells, a special class unique to amniote vestibular organs. Unlike previously studied hair cells, type I cells were found to have especially large numbers of voltage-gated potassium channels, but no sharp electrical tuning; such tuning would interfere with the signal's timing, of paramount importance in the vestibular system. Instead, the type I potassium channels facilitate a unique nonquantal synaptic transmission that delivers fast, linear, and temporally faithful head-motion signals to vestibular reflex pathways. This mechanism appears designed to stabilize vision and balance during rapid self-motions, a very different task than detecting sounds. In pursuing these questions, I think often of my debt to Jim and my good fortune to have been there as he set the stage.

Hippo-Mediated Control of Growth and Regeneration in the Inner Ear

Ksenia Gnedeva, *University of Southern California, Keck School of Medicine*

Individual Abstract: Unlike neonates, adult mammals cannot replenish lost hair cells, resulting in permanent hearing loss. This deficiency stems, in large part, from the failure of mature supporting cells to reenter the cell cycle and activate the transcriptional program necessary for the mechanosensory hair cell formation. Thus, targeting developmental pathways to “rejuvenate” supporting cells may unlock the latent regenerative potential in the mammalian inner ear. Using transcriptomic and epigenetic profiling, we assess molecular events associated with the establishment and maintenance of the quiescent postmitotic state in the inner ear and identified the Hippo pathway as a major driver of that process. We also utilized AAV gene delivery vectors and small molecule inhibitors of Lats kinases identified by us in a high-throughput screen to uncover the key role of the Hippo pathway in preventing proliferative regeneration in the mature mammalian inner ear. Our work expands the mechanistic understanding of how Hippo/Yap signaling controls mitotic quiescence and highlights important differences in the pathway regulation between vestibular and auditory sensory organs. We demonstrate that reactivation of Yap signaling after hair cell loss using either genetic or small-molecule approaches may promote mitotic regeneration of hair cells, a process thought to be permanently suppressed in the adult mammalian inner ear.

Coordinated Pathways for Mitotic Hair Cell Regeneration in the Avian Hearing Organ

Stefan Heller, *Stanford University School of Medicine*

Individual Abstract: Hearing loss is a widespread and irreversible condition in mammals due to the inability to regenerate auditory hair cells, whereas birds retain supporting cells capable of proliferative regeneration. Understanding how supporting cells control their cell cycle entry and produce new hair cells is essential for informing potential regenerative therapies.

Advances in single-cell RNA sequencing, combined with precise in vivo hair cell ablation models, have provided unprecedented resolution of the molecular events underlying avian hair cell regeneration. These studies enabled a comprehensive inventory of gene expression in individual cells and the identification of markers defining subgroups of hair cells and supporting cells. Temporal analyses revealed the sequence of cellular transitions during regeneration, showing how supporting cells progress from quiescence to proliferation and ultimately generate new hair cells.

Mechanistically, supporting-cell proliferation is governed by a coordinated network of signaling pathways. Proteolytic activation of the G-protein-coupled receptor F2RL1 initiates a cascade leading to HB-EGF shedding and EGFR–ERK signaling—events essential for S-phase entry. Our recent findings show that cAMP–PKA signaling is necessary for mitotic competence in vivo, which may either complement F2RL1 signaling or form a parallel cascade, while FGFB2 signaling, acting through the FGF receptor complex and phosphorylation of Smad2/3, represents another critical element. Together, these pathways converge to precisely regulate cell cycle progression in supporting cells and the production of new hair cells. Besides these pro-proliferative signals, spatially and temporally relevant downregulation of cell cycle inhibitors provides permissive control, aligning with differences in regenerative modes—mitotic proliferation versus direct transdifferentiation—between medial and lateral supporting cells.

Our current research focuses on the temporal and spatial interactions between these pathways, which we hypothesize ensure precise and balanced regeneration. Together, these findings

support an integrated mechanistic model in which multiple interactive pathways orchestrate supporting-cell proliferation and restoration of the sensory epithelium.

Working in Jim's lab offered both guidance and the freedom to explore, allowing me to pursue early single-cell experiments in the 1990s. Freedom and curiosity fueled scientific exploration, and discussing experiments that seemed technically impossible at the time planted seeds that bore fruit decades later. I am grateful for the opportunity to learn, experiment, and develop a mindset for tackling challenging questions under Jim's encouragement.

A Mentor Beyond Borders: The International Educational and Outreach Legacy of A.J. Hudspeth

Hiroshi Hibino, *Graduate School of Medicine, The University of Osaka*

Individual Abstract: Albert James Hudspeth was a global leader whose pioneering research and outstanding dedication to mentoring, education, and outreach left a lasting impact not only on the international scientific community but also on younger generations and children around the world. I had the honor of joining his laboratory from 1999 to 2002 as his first Japanese postdoctoral fellow. Over the 25 years since then, I have realized that his guidance has continued to shape not only my own scientific and personal journey but also those of many others around the world. His commitment to international scientific exchange was vividly demonstrated in 2001, when he came to Kobe in Japan as an invited speaker for the Japan Otological Society Annual Meeting. This was shortly after the September 11 attacks, when many international speakers canceled their travel. He came as planned, reflecting his strong belief in advancing science and education across borders, even in uncertain times. In 2018, he gave an inspiring special lecture at the Otological Society meeting at Osaka, and in 2024, despite his illness, he participated online in an international mechanobiology conference held in Sapporo, demonstrating his enduring commitment to education and collaboration. He also had a unique talent for communicating science to broad audiences. At 2009 Kyoto congress of the International Union of Physiological Sciences, his outreach lecture using a hair-bundle model fascinated students from elementary to high school. He also joined our kids' program in Osaka in 2018 and lectured for one week at Niigata University School of Medicine in 2013, leaving a lasting educational impact. Again, his mentoring, friendship, and leadership in global science and education inspired researchers and students worldwide. It is a great honor to celebrate his extraordinary contributions at this memorial symposium.

Don Caspary Honorary Symposium

7:15 p.m. - 9:15 p.m.

Ballroom B, Floor 3

Aging, Tinnitus and the Auditory Brain: A Symposium to Recognize the Contributions of Don Caspary

Daniel Llano, *University of Illinois*

Brandon Cox, *Southern Illinois University School of Medicine*

Multidisciplinary Approach to the Aging Auditory System: Behavioral Methods

Jeremy Turner, *Turner Scientific*

Individual Abstract: Dr. Caspary has approached important questions about the aging auditory system using an integrative, multidisciplinary, collaborative approach. This session will focus on the collaborative behavioral approaches he has employed and how those findings have helped to understand the functional impacts of electrophysiology and neurochemical findings in the aging auditory system.

The Role of the Auditory Cortex and the Inferior Colliculus in Mediating Neural Plasticity

Victoria Bajo, *University of Oxford*

Individual Abstract: The ability to modify the neural activity evoked by sensory experience is the basis of learning. Descending projections from cortical areas are among the most prominent pathways in any sensory system, suggesting their role in modulating subcortical processing according to the sensory context.

One of the main neural loops connects the primary auditory areas with the core auditory thalamus. Removing layer VI pyramidal neurons that project to the ventral division of the medial geniculate body impairs ferrets to discriminate mistuning tones, affecting their pitch perception that is essential to identify auditory objects within the scene analysis.

Another descending loop connects the auditory cortex with the inferior colliculus (IC) in the midbrain. Silencing optogenetically ArchT-expressing neurons in adult ferrets, we show that within-trial activity in the auditory cortex is required for training-dependent recovery of sound-localization accuracy following monaural deprivation. This learning-induced auditory plasticity requires the functional integrity of the corticocollicular pathway. The selective elimination of the large corticocollicular pyramidal cells in layer V impairs the ability of animals to adapt to changes in the binaural cue values produced by unilateral ear occlusion. In addition, activation of corticocollicular inputs in anaesthetised ferrets has a pronounced effect on both the spectral and spatial response properties of IC neurons, with reduced plasticity in the spatial response properties of IC neurons to a virtual unilateral earplug.

Recent results in our lab with chronic Neuropixels recordings in the Inferior Colliculus in awake ferrets performing a sound localization task, both with normal binaural inputs and following monaural occlusion, show that the behavioral localization performance of individual animals correlated with the neural activity in the IC under both hearing conditions, highlighting the role of the IC in experience dependent plasticity.

Those same neural circuits responsible for auditory plasticity in the adult brain are likely to be involved in the generation of tinnitus, a phantom auditory perception. We have optimised the use of gap-in-noise detection in humans and ferrets to identify temporal resolution impairment that might happen in tinnitus. Using optogenetic methods for manipulating brain activity, we are

exploring the changes that occur within the auditory cortex of ferrets experiencing tinnitus to find out whether those changes can be reversed to alleviate this condition in a clinical context.

My Career in One Line: Translating Don's GABA Work From Animals to People

Kelly Harris, *Medical University of South Carolina*

Individual Abstract: In 2006, I first asked Don to be a mentor on a clinician-scientist training award I was submitting. Don agreed and wrote in the letter: "I have a real interest in understanding the temporal deficits which develop in central auditory pathways consequent to presbycusis." Leading up to and since that time, Don's pioneering research has fundamentally advanced our understanding of how inhibitory neurotransmission, particularly mediated by GABA (γ -aminobutyric acid) shapes auditory processing across the lifespan. His decades of work provided critical insights into the role of GABAergic circuits in the central auditory system, especially within the inferior colliculus, cochlear nucleus, and auditory cortex.

Don was instrumental in developing the "Disinhibition Hypothesis of Presbycusis," demonstrating in animal models that age-related reductions in GABAergic inhibition within the auditory midbrain and cortex result in hyperexcitability, impaired signal-in-noise processing, and diminished temporal precision. By integrating electrophysiology, receptor pharmacology, and molecular assays, Don directly linked cellular mechanisms of inhibition to perceptual outcomes such as signal-in-noise perception—bridging basic auditory neuroscience with clinical perspectives on hearing loss.

As one of the few human subject researchers in the symposium today, I would like to share how much of my own work in humans has been directly inspired by Don's discoveries. In aging adults, we have observed parallel patterns between rats and humans including: peripheral decline leads to central hyperexcitability, degraded speech-in-noise recognition, and loss of GABA-mediated inhibition. This translational continuity underscores the enduring impact of Don's vision and the lasting influence of his contributions on our field.

Precision in Predictive Coding: The Role of GABAergic and Cholinergic Neurotransmission

Manuel Malmierca, *Cognitive and Auditory Neuroscience Laboratory (CANELAB), Institute of Neuroscience of Castilla y León (INCYL)*,

Individual Abstract: Dr. Caspary made a remarkable contribution to auditory neuroscience by pioneering the implementation of microiontophoresis in the auditory system. This technique enabled precise pharmacological manipulation of neural activity and opened new possibilities for studying the functional organization of auditory pathways. Building on his innovation, I applied microiontophoresis to investigate neuronal processing in the inferior colliculus, and I have since continued to use this approach in multiple studies, including those exploring predictive coding mechanisms in the auditory system. Using this method, we examined how GABAergic and cholinergic neurotransmission shape auditory processing, stimulus-specific adaptation, and prediction error responses in the inferior colliculus and auditory cortex. Notably, in the later stages of his career, Dr. Caspary himself also turned his attention to predictive coding, further

linking his groundbreaking methodological developments to emerging theoretical frameworks in auditory neuroscience. Both microiontophoresis and predictive coding not only connected our scientific interests but also made us close colleagues and collaborators over the years. In this presentation, I will share our recent data that integrate both microiontophoretic approaches and predictive coding analyses, and I feel deeply proud to contribute to this tribute honoring Dr. Caspary and his enduring scientific legacy.

Financial support was provided by projects PID2023-148541OB-I00, funded by MICIU/AEI <https://doi.org/10.13039/501100011033> and FEDER EU; Foundation Ramón Areces grant CIVP20A6616; Consejería de Educación, Junta de Castilla y León (SA218P23); and the strategic research programs of excellence from the Regional Government of Castile and León, co-funded by the ERDF Operational Programme (ref. CLU-2023-1-01) to MSM

The Yin and Yang of Ampa Glutamate Receptors at Auditory “Nerve” Synapses

Maria Eulalia Rubio-Valero, *University of Pittsburgh School of Medicine*

Individual Abstract: The integrated action of excitation and inhibition at the level of the cochlear nucleus is fundamental for high-fidelity auditory signal processing, setting the stage for more complex auditory tasks in higher brain centers. Donald Caspary long research interests in identifying key inhibitory players in synaptic integration has been fundamental in understanding normal and abnormal sound processing in the cochlear nucleus and beyond. His pioneer work positively impacted my research focused on unrevealing excitatory and inhibitory circuits and corresponding neurotransmitter receptors within the auditory brainstem.

We are interested in determining the role of AMPA glutamate receptors at the peripheral and central synapses of the auditory nerve on inner hair cells and cochlear nucleus, respectively. These studies provided important information that increased our understanding of the structural and molecular components and the function of auditory nerve synapses in normal hearing and hearing impairment. We have discovered striking sex differences in the hearing sensitivity in mice lacking the GluA3 AMPAR subunit (GRIA3 gene) (Rutherford et al., 2023; Pal et al., 2025). Furthermore, our studies also show differences in AMPA receptor subunit expression and hearing sensitivities in females and males during adolescence (Lozier et al., 2023, 2025). Fundamental sex differences in hearing have been observed in humans and mammals at sound onset in females and less vulnerability to noise-induced hearing loss in females than males. However, the reasons for these differences are unclear. Our studies imply the role of at least the GRIA3 gene in hearing sensitivities and hearing loss differences.

The Role of KCNQ Potassium Channels on Tinnitus and Hyperacusis

Thanos Tzounopoulos, *Univ. Of Pittsburgh*

Individual Abstract: I will discuss the role of KCNQ channels on tinnitus and hyperacusis and the development of new pharmacotherapies

Brain Mechanisms of Vocalization Processing Within and Beyond the Auditory System

Jeffrey Wenstrup, *Northeast Ohio Medical University*

Individual Abstract: Don Caspary has been a pioneer in the study of excitation and inhibition in the auditory system, in their modulation, and in their modification in aging and tinnitus. His impact on our field has been reflected in major themes of my laboratory for many years. One theme concerns the mechanisms underlying spectro-temporal integration associated with analyses of biosonar information in the echolocating mustached bat. Specialized neurons in the auditory brainstem, midbrain, and forebrain respond to the combination of two spectrally distinct elements in vocalizations to create a form of spectro-temporal integration called combination sensitivity, used for analyzing both sonar and social vocalizations. Employing the microiontophoretic approach pioneered by Don to assess excitatory and inhibitory function, we showed the critical role of glycinergic input in facilitatory interactions in the inferior colliculus and inhibitory interactions in nuclei of the lateral lemniscus. We further showed that low frequency-tuned glycinergic neurons in the medial nucleus of the trapezoid body and nuclei of the lateral lemniscus project non-tonotopically to mediate time-sensitive spectro-temporal integration. This work revealed novel roles for “inhibitory” neurons to underlie specialized responses to biologically relevant vocal signals. A more recent theme of my laboratory is less directly related to Don’s work but nonetheless enriched by it: the analysis of the emotional content in social vocalizations within and beyond the auditory system. Using mice and bats as models, our work describes integrative roles of the basolateral amygdala (BLA). Studies of vocal responses in the auditory midbrain and higher centers establish the types of vocal information available for amygdala processing. Within BLA, many neurons respond to social vocalizations in a highly selective manner, based on extracellular recordings. These neurons integrate a broader set of auditory inputs, as shown by post-synaptic potentials in intracellular recordings, to form the selective outputs. Responses to vocalizations but not to other acoustic stimuli depend on non-auditory sensory inputs that provide meaning to the vocal signals (e.g., olfactory cues). Additional contextual information, related to internal state, arises from neuromodulator inputs (especially acetylcholine and dopamine) to BLA that are activated differentially by emotional vocalizations associated with different behaviors. This work demonstrates the importance of excitatory, inhibitory, and neuromodulatory processes that have been major concerns of Don Caspary’s research program

Central Auditory Genes Related to Tinnitus and the Work of Donald Caspary

Allen F Ryan, *University of California, San Diego*

Individual Abstract: Background: Tinnitus is widely regarded as a phenomenon that arises in the response to cochlear damage, but moves to and is maintained in the central nervous system. Don Caspary has been a leader in the field of central auditory neural responses related to tinnitus. Within a research group studying the genetic underpinnings of hearing loss and tinnitus, we sought to identify genes related to either or both phenomena. In addition, I performed studies on the plasticity of neural responses in the central auditory pathway, to identify changes related to attention.

Methods: A GWAS for hearing loss and tinnitus was performed using data from the UK Biobank and the VA’s Million Veteran Program, with 596,925 subjects base. Hearing loss and tinnitus were identified by self-report plus audiometric data and CPTs. For attention studies, single unit responses were recorded from cochlear nucleus to auditory cortex in awake and

performing Rhesus monkeys. Responses were recorded when performing an auditory attention task versus passive rest periods.

Results: SNPs near the loci of 59 genes were found to be related to hearing loss or tinnitus. The majority of genes potentially related to hearing loss but not tinnitus were expressed in the cochlea, including both known deafness genes and novel genes. The great majority of genes potentially related to tinnitus, but not hearing loss, were expressed in the central nervous system. Many tinnitus-specific genes were also related to psychological disorders. Studies of single unit plasticity noted similar attention-related variation from the cochlear nucleus through the auditory cortex.

Conclusions: The results of our GWAS are consistent with many epidemiological, clinical and basic studies implicating a central origin of tinnitus. They add potential genetic substrates to the characteristics of tinnitus, including many drugable target including GABA-related receptors. The GWAS results also validate the tinnitus work of Don Caspary, which highlights the role of GABA in this phenomenon. Single unit results confirmed extensive efferent control of central auditory responses.

Donald Caspary: I have known Don for about 40 years, from meetings attended, the Tinnitus Research Consortium, dinners shared and runs together. One of my earliest and most vivid memories is from a conference on the English countryside, where we escaped the meeting to run through beautiful fields, over stiles, dodging cow and sheep patties. Although I have never collaborated with Don, he has often invited me to his lab dinners at the ARO, where we shared food and friendship.

Wednesday, February 11, 2026

Podium 18: Auditory Prostheses #2

8:00 a.m. - 10:00 a.m.

Meeting Room 209ABC, Floor 2

Moderators: Julie Arenberg and Seba Ausili

Treating Hearing Loss: Cochlear Implantation Versus Gene Therapy

8:00 a.m. - 8:15 a.m.

Fan-Gang Zeng^{*1}, Jieyu Qi², Chen-Chi Wu³, Yilai Shu⁴, Renjie Chai⁵

¹*University of California Irvine*, ²*Beijing Institute of Technology*, ³*National Taiwan University Hospital*, ⁴*Eye and ENT Hospital, Fudan University*, ⁵*Southeast University*

Background: Gene therapy has recently restored natural audibility in humans with OTOF-gene mutations. This biological restoration of hearing is different from cochlear implantation (CI) that produces artificial hearing via electric stimulation of the auditory nerve. While current result marks a significant milestone in treating genetic forms of deafness, several central questions remain. First, is gene therapy for hearing loss safe and stable? Second, how effective is gene therapy in terms of improving audibility and functional hearing at both individual and population levels? Third, what must gene therapy achieve for it to compete or possibly replace CI for patients with monogenic forms of deafness?

Methods: To address these questions, we summarize all publicly available data from current human clinical trials. In addition, we have collected perceptual data in patients who received gene therapy and compare their performance to those with CI and normal hearing.

Results: In three published trials, 21 DFNB9 patients show 244 grade I/II adverse events (AEs), 2 grade III AEs and no serious AEs. The average gene therapy effect is substantial (52.4-dB improvement from baseline complete deafness), rapid (0.74-month time constant), and stable over up to two years – the longest observation so far. However, individual outcomes vary from restored normal audibility (≤ 20 dB HL) to severe hearing loss (≥ 80 dB HL). Suprathreshold temporal processing shows a similar range of variation from normal temporal gap detection thresholds (~ 2 ms) to severe auditory neuropathy (> 20 ms). On average, patients with gene therapy perform similarly to those with CI, with some performing at nearly normal level.

Conclusions: Gene therapy for OTOF mutations is safe, rapid and stable in terms of restoring natural audibility, a feat that has never been achieved by cochlear implants. However, the individual variability in gene therapy is large and robust evidence for restoring critical auditory functions such as temporal processing remains lacking. Due to the well-established evidence for quality-of-life improvements and cost-effectiveness, cochlear implantation will remain the primary intervention for treating severe-to-profound hearing loss in the near term, whereas gene therapy will likely become an alternative to cochlear implantation in monogenic forms of deafness in the future.

Cochlear Implants More Than Stimulators? Chronic Electrochemical Sensing in Rats

8:15 a.m. - 8:30 a.m.

Aline R. Xavier¹, Stefan Reinelt², Susan Arndt³, Stefan J. Rupitsch², Andreas Weltin², Jochen Kieninger², Nicole Rosskoth-Kuhl^{1*3}

¹Neurobiological Research Laboratory, Section for Experimental and Clinical Otology, Medical Center - University of Freiburg, Medical Faculty, University of Freiburg, Freiburg, Germany, ²IMTEK – University of Freiburg, Germany, ³Medical Center - University of Freiburg, Faculty of Medicine, Freiburg, Germany

Background: Cochlear implants (CIs) are the most successful neural implants worldwide, restoring hearing by directly stimulating the auditory nerve. Traditionally, these devices have been used as stimulators, but recent research has opened the possibility of their application as sensors. Weltin et al. (2022) developed an innovative electrochemical method for measuring dissolved intracochlear oxygen in acutely cochlear-implanted rats. This novel sensor function enables monitoring of the implant's microenvironment and electrode status and promises viable insights into the development of inflammation reactions after cochlear implantation, as well as additional monitoring functionality besides impedance measurements. Building on this, we here investigate if CI electrodes are long-term stable in vivo sensors and whether sensor measurements can be performed independently of the activity status of the CI user.

Methods: Deaf Wistar rats were bilaterally implanted with CIs and underwent binaural sound lateralization training for up to six months, with all animals showing excellent hearing performance. For the sensor measurements, CIs were connected to a potentiostatic measurement setup, and CI sensing was performed at different time points, either in anesthetized or awake animals. The experimental protocol included preconditioning to stabilize the electrode surface and electrode characterization using cyclic voltammetry. The sensing measurement combined amperometric and potentiometric methods to detect the dissolved oxygen in the cochlea in real-time.

Results: Over months of chronic implantation, it was possible to reproducibly record the sensor signal and sense intracochlear oxygen levels. The oxygen reduction reaction (ORR) current and open circuit potential (OCP), which were extracted from electrochemical measurements, showed stable and repeatable responses to oxygen supply. Behavioral lateralization training and electrode impedances were not affected by repeated CI sensing, demonstrating that performing (repeatedly) electrochemical methods does not affect CI function. Furthermore, sensing was possible independently of the animal's wakefulness.

Conclusions: We have demonstrated that CIs can successfully be used as long-term stable in vivo sensors, enabling periodic electrochemical measurements without affecting the animal's behavioral performance. This novel functionality of CI electrodes offers great potential for long-term investigations of the condition of CI electrodes over time and provides insights into the biochemical microenvironment of the cochlea following implantation, such as the foreign body response. The application of this method may contribute to improving implant longevity.

A Dual Approach Towards Detecting in Situ Cochlear Implant Trauma: Optical Coherence Tomography and Electrochemical Sensing

8:30 a.m. - 8:45 a.m.

Lore Kerkhofs*¹, Jolan Wellens², Tristan Putzeys³, Olivier Deschaume², Myles Mc Laughlin⁴, Carmen Bartic², Nicolas Verhaert⁴

¹KU Leuven, ²KU Leuven, University of Leuven, Leuven, Belgium, ³ExpORL, KU Leuven, University of Leuven, Leuven, Belgium, ⁴ExpORL, KU Leuven

Background: Cochlear implants (CI) are one of the most effective treatments for severe hearing loss and are increasingly implanted in patients with some intact residual hearing. Preserving residual hearing drastically improves speech perception and CI performance. However, insertion trauma often destroys residual hearing. Currently, there is a lack of methods capable of detecting insertion trauma intra-operatively.

Methods: A dual approach combining optical coherence tomography (OCT) and CI electrodes, modified to function as H₂O₂ sensors, was used to detect intracochlear trauma in an in-vivo gerbil model. The study included 9 cochleae in which trauma was induced and 5 control (no-trauma) cochleae. Contrast-enhanced computed tomography (CECT) on isolated cochleae was used as a benchmark for both trauma classification and quantification.

Results: Using OCT, we could visualize intra-cochlear trauma in the hook region, including basilar membrane ruptures and Osseous spiral lamina (OSL) fractures. OSL trauma is quantifiable through analysis of surface smoothness variations relative to pre-trauma OSL. H₂O₂ concentrations, as readout by the sensor, are correlated with trauma volume ($p = 0.006$) and trauma scale ($p = 0.035$). A receiver operating characteristic curve was established for the detection of intracochlear trauma (Cutoff level = 42 μ M, Area under curve = 0.85).

Conclusions: Intracochlear trauma in the hook region can be detected in vivo using OCT. Trauma leads to an upregulation of H₂O₂. Our sensor can be used as an effective detection method for intracochlear trauma. This study establishes a foundation for advanced CIs that integrate real-time monitoring of the intracochlear environment.

Rehabilitation for Help-Seeking Veterans With Functional Hearing Difficulties: A Randomized Controlled Trial

8:45 a.m. - 9:00 a.m.

Melissa Papesh*¹, Matthew Hamilton-Sutherland¹, Jay Vachhani¹, Sambith Rout¹

¹Department of Veterans Affairs

Background: Approximately 10% of Veterans who seek care from VA audiologists for hearing difficulties are found to have normal hearing sensitivity (Billings et al., 2018). It is typically assumed that these patients' primary auditory difficulties revolve around speech understanding in noise and most likely to stem from neurological injuries or conditions (e.g., brain injuries, neurotoxic exposures, etc.) or possibly mental health concerns. Such difficulties have been referred to functional hearing difficulties (FHD) given the impact on patients' daily functioning despite normal hearing sensitivity. The goal of this presentation is to (a) learn more about Veterans with FHD, and (b) conduct a randomized controlled trial to evaluate the benefit of fitting low-gain hearing aids (LGHA) in this population.

Methods: Participants have normal to near-normal hearing sensitivity and scores indicating moderate to severe perceived hearing handicap to meet study inclusionary criteria. They undergo hearing-related testing (standard and high-frequency audiometry, otoacoustic emissions, acoustic reflexes, and auditory processing evaluation), a cognitive test battery, questionnaires probing mental health conditions, personality assessment, tinnitus, sound sensitivity, and previous medical history, as well as an interview to learn more about their

lives and their hopes regarding auditory rehabilitation. Participants are then randomly assigned to one of three groups to receive either communication counseling (CC) alone or in combination with either LGHA or both LGHA and a remote microphone (RM). Perceptual, self-report, and electrophysiological measures retested at 2 months following intervention.

Results: Preliminary results are based upon 13 currently enrolled participants. These results indicate a range of performance on tests of central auditory processing across participants with some meeting ASHA criteria for auditory processing disorder while others score in the normal range all test measures. The most commonly reported communication difficulties include work settings (conversing with clients, coworkers, and managers) followed by social settings (understanding partners and family members in various settings). Patients with functional hearing difficulties (FHD) are not more likely to score more highly than the general population on measures of neuroticism nor more poorly on measures of cognition. Regarding response to intervention, participants who receive LGHA tend to wear them consistently and often prefer higher amplification settings than those prescribed by NAL-NL3 standards based upon their pure-tone sensitivity. Improved performance in noisy background settings (vehicle noise, restaurant noise, competing talkers) is found only when using the FM system despite participants reporting benefit from use of hearing aids in their daily lives even when not using or provided with FM technology.

Conclusions: LGHAs may be a highly effective means of ameliorating communication difficulties in patients with FHD. Greater emphasis should be placed on patient self-report measures rather than basing clinical recommendations on performance-based test results alone.

Extracochlear Electric Stimulation — Towards Non-Invasive Hearing Restoration and Diagnostics

9:00 a.m. - 9:15 a.m.

Robert Hart¹, Patrick Hinz¹, Constantino Dragicevic², Mechthild Meierott², Benjamin Krüger¹, Waldo Nogueira¹

¹Hannover Medical School, ²Institute of Neuroscience, Universitat Autònoma de Barcelona, Spain

Background: In individuals with severe-to-profound hearing loss, hearing aids often cease to be effective, leaving cochlear implantation (CI) as the main alternative. However, CI surgery carries the risk of damaging residual hearing, which is known to substantially enhance speech perception in CI users when preserved. Protecting residual hearing is also crucial for enabling future rehabilitation via emerging technologies. We propose a novel approach based on extracochlear electric stimulation (EES), in which electrical stimulation is delivered via an electrode outside the cochlea, potentially avoiding invasive insertion of an intracochlear array. Such a system could implement electro-acoustic stimulation (EAS), using acoustic amplification for the residual low-frequency range while electrically stimulating non-functional cochlear regions. Moreover, the interaction between acoustic and electric stimulation thresholds can provide diagnostic information, particularly for assessing low-frequency residual hearing, where current diagnostic tools are limited.

Methods: The conducted study assesses the effectiveness of EES via an active ear canal electrode combined with different reference electrode placements. We aim to determine optimal stimulation configurations and their ability to elicit auditory sensations across frequencies from 250 Hz to 4 kHz. Participants include: 5 normal-hearing adults, 5 adults

with significant high-frequency hearing loss (> 1 kHz), and 5 cochlear implant users. Electrical stimuli are 500 ms sinusoidal signals. Participants report perceived loudness on a 0-10 scale, and side effects are rated on a 0-10 scale with descriptive categorization. Threshold and maximum comfortable level are recorded for each condition, and possible side effects are characterized in detail.

Results: Preliminary findings indicate that EES can elicit auditory sensations across all participant groups, though loudness growth and side effect profiles vary with reference electrode placement and frequency. Early results suggest measurable differences in thresholds between the tested configurations, with contralateral reference placements being the most effective in terms of evoked hearing sensation. While normal-hearing subjects perceived the auditory sensation mainly as a sinusoidal pure tone, hearing impaired and deafened subjects often reported a broader range of mixed sounds. In general, frequencies below 1kHz appeared to be more effective for any sound sensation across all participants. The reported side effects vary from frequency and condition but often include a stinging and tingling around the electrodes, limiting the possible hearing outcome.

Conclusions: This study explored EES as a potential non-invasive alternative to CI stimulation, with applications in both rehabilitation and diagnostics. Hearing sensation was achieved in all three groups, however, side effects still remain a significant issue and solutions to minimize these have to be explored extensively. When further refined, the acoustic-electric interaction may serve as a hearing aid device and as a sensitive tool for assessing residual low-frequency hearing. Our findings inform the development of future EES-based devices, bridging the gap between traditional hearing aids and cochlear implants.

Neural Feedback From Auditory Brainstem Implant Stimulation

9:15 a.m. - 9:30 a.m.

Mahan Azadpour^{*1}, Rahul Sinha¹, Nicole Capach¹, Megan Eitel¹, William Shapiro¹, J. Thomas Roland¹, Mario Svirsky¹

¹*New York University Grossman School of Medicine*

Background: Auditory brainstem implants (ABIs) restore hearing for individuals with damaged or abnormal cochlear structures or auditory nerves who cannot benefit from cochlear implants. While ABIs are similar to cochlear implants in terms of hardware, software, and stimulation strategies, speech perception outcomes with ABIs are often much poorer. This discrepancy likely stems from the complex electrode-neural interface of ABIs. The ABI electrode paddle targets the surface of the cochlear nucleus within the brainstem, which is a complex structure with diverse neuron types and biophysical properties. Many neuron types in the cochlear nucleus may not effectively convey speech information from ABI stimulation.

Methods: This study aimed to investigate neural activity in the brainstem evoked by ABI stimulation and assess whether feedback from the primary stimulated neurons could inform more effective ABI stimulation strategies. Electrically evoked compound action potentials (ECAPs) were recorded from ABI stimulation sites using the device's voltage recording capabilities. ECAP waveform morphology and forward masking patterns were analyzed to characterize responses from neurons stimulated by ABIs.

Results: Our findings revealed significant variability in ECAP measures across ABI users and between electrodes within individual users, reflecting the diverse neural populations stimulated by ABI electrodes. ABI ECAP waveforms often differed from the typical

negative-positive peaks observed in cochlear implants, with forward masking recovery times sometimes exceeding those seen in cochlear implants by an order of magnitude. Spatial forward masking patterns indicated relatively localized neural excitation within the brainstem, with masking effects decreasing steeply as inter-electrode distances increased, although less systematically than in cochlear implants. Notably, ABI electrodes with similar neural response characteristics often clustered together within an ABI paddle.

Conclusions: The variability in ABI ECAP indicates the need for personalized approaches to optimize ABI stimulation, taking into account the biophysical properties of the primary stimulated neurons. The ECAP waveform morphology and masking patterns provide insights into the responsiveness of cochlear nucleus neurons to ABI stimulation. Feedback from the primary stimulated neurons could provide crucial information for tailoring ABI stimulation strategies to improve hearing and speech perception outcomes.

The UmboMic: Biocompatibility and Testing of a Middle Ear Microphone for Fully Implanted Cochlear Implants

9:30 a.m. - 9:45 a.m.

Emma Wawrzyniek¹, John Zhang¹, Julie Arenberg², D. Bradley Welling³, Ioannis Kymissis⁴, Elizabeth Olson⁴, Jeffrey Lang¹, Hideko Heidi Nakajima⁵

¹Massachusetts Institute of Technology, ²Massachusetts Eye and Ear Infirmary, Eaton Peabody Laboratory, ³Harvard/Massachusetts Eye and Ear, ⁴Columbia University, ⁵Harvard Medical School, Mass. Eye and Ear Infirmary

Background: Realizing a fully-implanted cochlear implant depends on the development of an appropriate microphone. We present advancements of the UmboMic, a piezoelectric sensor and amplifier implanted in the middle ear to detect motion of the umbo. The UmboMic functions as a microphone by transducing the umbo's vibration into an electrical signal. Our prior experiments on the bench and in human cadaveric ears demonstrate the UmboMic's excellent signal-to-noise ratio and linearity over a wide dynamic range. We now report a new method of UmboMic fabrication, which achieves a fully biocompatible sensor. Despite the use of new materials, the biocompatible UmboMic has similar performance on the bench. Finally, we discuss longevity testing of the UmboMic sensor via accelerated aging experiments, and methods for characterizing the device's resilience to chronic implantation.

Methods: UmboMic sensors are fabricated in the cleanroom at MIT.Nano using photolithography and thin-film deposition techniques. The active layers of the sensor are two polarized sheets of piezoelectric PVDF. Finished UmboMic sensors are tested in an electrically- and acoustically-isolated sound chamber. UmboMic sensors are first evaluated on the bench using an "artificial umbo," and next tested in human cadaveric temporal bones.

Accelerated aging of the UmboMic sensors is performed by suspending the device in phosphate buffer solution at 65°C. The 10-degree rule governs the accelerated rate of aging at higher temperatures. Aged sensors can be evaluated for robustness by measuring input impedance and performing energy-dispersive X-ray spectroscopy (SEM) imaging.

Results: Bench and cadaveric experiments demonstrate that the previous non-biocompatible UmboMic has a low noise floor of approximately 62E-18 C from 200 Hz to 20 kHz, and a maximum sensitivity of about 230E-15 C/Pa at 2 kHz. The UmboMic also shows a linear response over a dynamic range of 35 to 115 dB SPL. The fully-biocompatible UmboMic

sensors perform similarly to the previous non-biocompatible versions on the bench, with comparable sensitivity, noise floor, and linearity.

Measurements of UmboMic sensors via an impedance analyzer results in an input impedance matching the analytical circuit model of the sensor. The input impedance is a predictable metric for tracking changes in the sensor's electrical properties as a result of aging. After aging, imaging with EDS allows for identification of material layers in the UmboMic sensor and changes that may occur. Preliminary aging of PVDF indicates no change in sensitivity.

Conclusions: This work demonstrates the success of an UmboMic sensor comprising fully biocompatible materials. Developing a biocompatible UmboMic sensor is critical to achieving a chronically safe fully-implanted cochlear implant. Future work on the UmboMic will include long term accelerated aging studies with continuous evaluation of the robustness of the UmboMic sensor, providing feedback for changes in fabrication techniques. We will also explore thin film encapsulants for increased device robustness.

Open-Set Speech Perception With the Auditory Brainstem Implant in Patients With Neurofibromatosis Type 2-Related Schwannomatosis

9:45 a.m. - 10:00 a.m.

Camelia Saber*¹, Sonja Poe¹, Samantha Shave¹, M. Christian Brown¹, Christine L. Carter², Barbara S. Herrmann², Daniel J. Lee²

¹*Eaton Peabody Lab, Massachusetts Eye and Ear, Boston, MA, USA*, ²*Massachusetts Eye and Ear, Boston, MA, USA*

Background: Few auditory brainstem implant (ABI) users achieve open-set word recognition using auditory input alone, unlike the majority of cochlear implant (CI) users. Previous studies have found that ABI users with better speech outcomes tend to have lower charge thresholds and more active electrodes than those with poorer performance, suggesting a smaller distance to the cochlear nucleus complex (CN). This study investigates outcomes in exceptional Neurofibromatosis type 2 (NF2)-related schwannomatosis ABI users. We examined the processor programming, speech perception testing, and the ABI array positioning on postoperative imaging.

Methods: Exceptional ABI users were selected from ABI users who underwent surgery and received postoperative clinical care at our institution to ensure complete data. Speech perception outcomes were assessed using the closed-set Early Speech Perception (ESP) test and the open-set Consonant-Nucleus-Consonant (CNC) monosyllable word recognition test. Demographic, medical, and audiological data were extracted from clinical records of adult NF2 ABI users with CNC scores above 20% correct. The number of active electrodes, charge thresholds, and pitch perception patterns across the electrode array were analyzed. The relationship between charge thresholds and ABI array positioning using three-dimensional (3D) postoperative computed tomography (CT) reconstructions was also explored.

Results: Two patients with NF2-related schwannomatosis met the inclusion criteria. Both developed single-sided deafness following vestibular schwannoma (VS) resection, progressing to bilateral deafness before receiving a Cochlear™ Nucleus®ABI541 during contralateral VS surgery. Both patients used their ABI full-time and achieved CNC scores of 40% between 7 to 8 years after activation. They exhibited similar charge thresholds and pitch perception rankings across their arrays. Although CT reconstructions showed that the arrays did not overlap in position relative to surrounding bony structures, the pitch perception

rankings overlapped in 3D space when rotated according to the angle of the array as measured by CT reconstruction.

Conclusions: Two NF2 ABI users with exceptional speech performance were full-time device users for over 7 years. Charge thresholds and pitch perception profiles were comparable when adjusted by the 3D positioning relative to the skull base. Charge thresholds may indicate array placement relative to the CN, while pitch perception patterns may reflect the rotation relative to the tonotopic arrangement of the CN. As the array position relative to bony landmarks does not always indicate placement relative to the CN, CT may prove to be more valuable when combined with charge thresholds and pitch sensations.

Podium 19: Next-Gen Approaches in Ear Gene Therapy: Tools, Targets, and Regenerative Pathways

8:00 a.m. - 10:00 a.m.

Ballroom B, Floor 3

Moderators: Saaïd Safieddine and Christopher Cunningham

Phenotypic Characterization and Application of CRISPR-Cas9-Based Genome Editing to a Novel Mouse Model of DFNA20/26 Further Advance Our Understanding of Gamma-Actin Structural Role in Hair Cells

8:00 a.m. - 10:00 a.m.

Mhamed Grati¹, Kevin Isgrig¹, Jianliang Zhu¹, Hongjun Wang¹, Yasuko Ishibashi², Inna A. Belyantseva³, Keith Abbey¹, Zeynep Ozgur¹, Samantha C. Lau¹, Karen Friderici⁴, Mei Zhu⁴, Erich Boger⁵, Robert Morell⁵, Meghan Drummond⁶, Thomas B. Friedman³, Wade W. Chien⁷

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Background: Sensorineural hearing loss is a common disorder which affects the world's population. Previous inner ear gene therapy studies have shown promising results in improving auditory function in various mouse models of sensorineural hearing loss. While many studies focus on non-syndromic autosomal recessive hereditary hearing loss (DFNB), non-syndromic autosomal dominant hereditary hearing loss (DFNA) may be a better candidate for inner ear gene therapy translation due to the usual later onset and slower

progression of hearing loss of dominant variants. A slow progressive loss of hearing offers a wide therapeutic window for treatment. DFNA20/26 is associated with variants in the Gamma-actin gene (ACTG1) which is abundantly expressed in the inner ear. In this study, we characterized a novel Actg1P264L/P264L mouse model of human DFNA20/26 and then applied CRISPR-based genome editing to improve auditory function.

Methods: We characterized outer and inner hair cell stereocilia defects of Actg1P264L/P264L mice using scanning electron microscopy (SEM) and immunofluorescence techniques at several maturation stages. Guide RNAs (gRNAs) for Cas9 nuclease were designed and validated in vitro to preferentially target the P264L mutation-encoding allele. The gRNA with the best sensitivity and specificity to target and edit the P264L mutation-encoding allele was delivered via adeno-associated virus (AAV2-7m8 serotype) along with Cas9 nuclease into neonatal Actg1P264L/P264L inner ears using the posterior semicircular canal approach. Hearing was evaluated at 1 and 2 months post-delivery using ABRs and DPOAEs.

Results: Actg1P264L/P264L mutant mice developed early postnatal deformations of the OHC apical surface and of the V-shaped stereocilia bundle architecture, which correlated with a significant decrease in α -actin at the apical lateral belt, and showed splitting of maturing OHC stereocilia bundles that correlated with the fragmentation and structural defects in the cuticular plate. Plasma membrane blebbing was also observed at the tips of maturing OHC mechanotransducing stereocilia that was previously correlated to MET complex anomalies. In addition, we observed stereocilia row identity anomalies in Actg1P264L/P264L IHC stereocilia bundle similar to those previously observed as a characteristic phenotype of several mouse models mutant in MET complex proteins.

We found that Actg1P264L/P264L mutant mice that were treated with CRISPR gene-editing-based therapy showed improved outer hair cell stereocilia morphology and improved ABR thresholds at ~P30 compared to untreated mutant mice. However, this improvement in ABR thresholds was not sustained at ~P60.

Conclusions: Our results demonstrate that α -actin is crucial for the early shaping and integrity of OHC V-shaped bundle architecture via its integration into their apical lateral belt and their cuticular plate. α -actin is also involved in maturation and stability of the MET complex in both cochlear OHC and IHCs. We also found that CRISPR-based genome editing was able to improve the stereocilia morphology and slow the deterioration of auditory function in Actg1P264L/P264L mice.

Novel Antisense Exon Skipping Therapy for USH2A

8:00 a.m. - 10:00 a.m.

Stephanie Mauriac^{*1}, Yu-Han Huang¹, Camille Martin¹, Jiahe Jin¹, Sydney O'Malley¹, Carl Nist-Lund¹, Jiyeon Lee¹, Jennifer B Phillips², Jeremy Wegner², Monte Westerfield², Karl Koehler¹, Timothy Yu¹, Gwenaëlle Geleoc¹

¹*Boston Children's Hospital, Harvard Medical School*, ²*Institute of Neuroscience, University of Oregon*

Background: Usher syndrome (USH) is the most common genetic cause of combined deafness and blindness. Mutations in USH2A account for half of USH cases. The size of the USH2A coding sequence (~15.6Kb) prevents the use of adeno-associated virus (AAV)-based gene replacement therapy, because it exceeds the capacity of single and even dual AAVs. To

circumvent this limitation, we are developing antisense oligonucleotide (ASO) strategies to correct hearing and vision loss in USH2A patients. Here we are targeting an out-of-frame founder mutation in USH2A, identified in patients at Boston Children's Hospital, and a major cause of USH2A in the French-Canadian population. We hypothesize that dual exon skipping of the affected region will restore the proper reading frame and result in expression of slightly shorter, yet still functional USH2A protein (usherin).

Methods: We designed ASO sequences based on computational analyses of cis-regulatory sequences predicted to impact splicing. Candidate ASOs were synthesized with modified 2'-O-methyl nucleotides and phosphorothioate backbones. ASOs were either (1) transfected into retinoblastoma cells (WERI-RB1) or (2) applied gymnotically to inner ear and retinal organoids derived from human mutant, isogenic or healthy iPSC lines. We performed RT-PCR to validate ASO-induced USH2A dual-exon skipping. To achieve sustained exon skipping and bypass the need for repeated injections required by ASO-based strategies, we also tested a vectorized form of the most effective ASO sequences, taking advantage of the vectorized U7snRNA delivery system. At the protein level, in silico analysis was performed using AlphaFold2 to predict the 3D structure of both wild-type and pseudo-USH2A protein. To validate functional recovery with the shorter transcript, we generated two sets of zebrafish, one mimicking the mutation and the other with the dual exon deletion. We performed phenotypic vision assessments of the fish models.

Results: We demonstrated successful single and dual exon skip with different combinations and dosages of ASOs in RB1 cells and in human organoids. The exon skipping strategy led to an in-frame internal deletion of 105 amino acids of 5202 amino acids. This deletion excised a single fibronectin domain. In silico analysis demonstrated preservation of protein folding suggesting preservation of usherin function. Indel zebrafish had altered visual phenotypes. The dual exon deletion fish model is currently being assessed. We are also assessing vectorized ASOs in control and mutant organoids.

Conclusions: ASO therapy offers a promising approach to target genetic disorders, especially those associated with large genes, including USH2A. ASOs are simple to customize for different targets, inexpensive to manufacture, and can be efficiently delivered without special formulation. By demonstrating the high efficiency and safety of this approach, we aim to bring this novel antisense therapy from the bench to the bedside of USH2A patients.

Adeno-Associated Viral Vector Based Cochlear Gene Therapy for Hearing Loss in Norrie Disease

8:00 a.m. - 10:00 a.m.

Aara Patel^{*1}, Oliver Coombe-Tenant², James Arwyn-Jones², Carlos Aguilar³, Deepak Chandrasekharan², Alva Supit², Sandrine Vitry⁴, Aziz El Amraoui⁴, Michael Bowl⁵, Jane C. Sowden²

¹University College London, ²UCL Great Ormond Street Institute of Child Health, University College London, and NIHR Great Ormond Street Hospital Biomedical Research Centre, London, United Kingdom, ³UCL Ear Institute, ⁴Institut Pasteur, Institut de l'Audition, ⁵UCL Ear Institute, University College London

Background: Norrie disease is an X-linked recessive dual sensory disorder, caused by NDP gene mutation. Affected children have congenital blindness and progressive sensorineural hearing loss from childhood. To develop a therapy, we use a mouse model of Norrie disease

(Ndp^{tm1Wbrg}; Ndp-KO/C57BL/6), which recapitulates Norrie disease pathology. In the Ndp-KO cochlea, early vascular abnormalities and loss of vascular barrier function are followed by a loss of cochlear hair cells and progressive loss of hearing. NDP encodes norrin, a secreted signalling molecule that acts on target cells by binding to a FZD4/LRP5/6/TSPAN12 receptor complex and stabilizing intracellular β -catenin. In previous work we showed that the Norrie pathology was ameliorated by constitutive activation of β -catenin in vascular endothelial cells, or intravenous (systemic) delivery of an adeno-associated viral vector (AAV) carrying the human NDP coding sequence (PMID: 37642150, 39585982). In this study we aim to test a clinically applicable vector and delivery route in the Ndp-KO Norrie disease model that could be used to treat progressive hearing loss in individuals with Norrie disease.

Methods: A therapeutic construct, consisting of the human NDP coding sequence under the control of a strong promoter, was packaged into AAV9 capsids (AAV9.NDP). TopFlash assays of β -catenin signalling were performed to test production of functional norrin by the construct. AAV9.NDP was injected into the perilymphatic space of the cochlea of Ndp-KO mice via the round window membrane at postnatal day 22 (P22) and P30; timepoints that correspond to childhood and young adulthood in Norrie disease patients and are clinically relevant for treatment. At 2 months of age auditory function was assessed by recording auditory brainstem responses (ABR), hair cell survival was quantified by imaging cochlear whole mounts and vector biodistribution in peripheral organs was assessed.

Results: In vitro TopFlash assays showed the AAV9.NDP gene therapy produced norrin and activated β -catenin signalling. Following cochlear delivery of the AAV9.NDP therapeutic construct, localization of the vascular tight junction protein Cldn5 was restored in lateral wall vasculature. Treatment at P22 completely preserved outer hair cells and hearing, while treatment at P30 also showed significant preservation of outer hair cells and partial preservation of ABR thresholds. Notably, cochlear AAV9.NDP gene therapy prevented disease progression even when administered after the onset of pathological changes to the cochlear vasculature in Ndp-KO mice.

Conclusions: The AAV.NDP gene therapy vector produces functional norrin and ameliorates hearing loss in the Ndp-KO Norrie disease model after administration to the cochlea. This study supports the feasibility of a clinical treatment for progressive hearing loss in Norrie disease patients during childhood and young adulthood.

Acknowledgements: Supported by Great Ormond Street Hospital Children's Charity, the Royal National Institute for Deaf People, Fondation Pour l'Audition, the Norrie Disease Foundation, UCL Therapeutic Acceleration Support scheme.

The Evolutionary Edge in Gene Therapy for Mammalian Hearing: Phylogenetic Lessons From a Clarin-2 Deficient Model

8:00 a.m. - 10:00 a.m.

Sandrine Vitry^{*1}, Aïnoa Malaterre¹, Clara Mendia¹, Clara Mendia¹, Emilia Wysocka¹, Wendy Nguyen¹, Amandine Haddad¹, Mûge Senarisoy¹, Michael Bowl², Aziz El-Amraoui¹

¹*Institut Pasteur, IHU reConnect, Institut de l'Audition*, ²*UCL Ear Institute, University College London*

Background: Evolutionary specialization of the mammalian inner ear underlies exceptional auditory sensitivity but may also constrain therapeutic strategies. We investigated how evolutionary divergence and gene sequence variation influence outcomes of gene therapy in a *Clarin-2* deficient (*Clrn2*^{-/-}) model of progressive hearing loss.

Methods: *Clrn2*^{-/-} neonates were injected in the inner ear with AAV-PHP.eB vectors carrying *Clrn2* sequences from species positioned at crucial phylogenetic nodes of the evolutionary tree: zebrafish (*Danio rerio*), frog (*Xenopus laevis*), lizard (*Anolis carolinensis*), chicken (*Gallus gallus*), platypus (*Ornithorhynchus anatinus*) and human. Chimeric *Clrn2* constructs were also tested. Hearing rescue was assessed one month later and inner ears collected for qPCR, confocal or scanning microscopy.

Results: Audiometric tests revealed varying degrees of hearing recovery, with human, platypus, chicken and frog *Clrn2* orthologs outperforming zebrafish and lizard ones, at comparable transgene expression levels. Histological exploration will show if organ architecture preservation is correlated with hearing scores. Importantly, cross-species amino acid comparisons showed that even subtle sequence differences in *clarin-2* markedly altered therapeutic success, with two domains potentially crucial for its biological function: the intracellular C-terminal PDZ binding domain and the first transmembrane domain.

Conclusions: By integrating phylogenetic analyses with functional rescue studies, our work highlights how evolutionary differences may guide sequence optimization for gene therapy.

Specific Promoter Aav Toolbox for Cross-Species Targeting of Cochlear Hair Cells and Gene Therapy

8:00 a.m. - 10:00 a.m.

Liyan Zhang^{*1}, Yicheng Lu¹, Ling Lu², Jieyu Qi³, Renjie Chai¹, Fangzhi Tan¹

¹*Southeast University*, ²*Zhongda Hospital Southeast University*, ³*Beijing Institute of Technology*

Background: Cochlear hair cell (HC) dysfunction is a leading cause of hereditary hearing loss, accounting for approximately 50% of cases. Age-related hearing loss is one of the most common sensory disorders among older adults, and is also predominantly caused by hair cell damage. AAV-mediated gene therapy represents the most promising strategy for hearing restoration. However, the highly heterogeneous cellular composition and unique microenvironment of the cochlea pose significant challenges for targeted gene delivery into HCs. Therefore, developing hair cell targeted gene therapies with high specificity and safety holds significant scientific and commercial value.

Methods: To develop cochlear HC-specific AAV tools, we constructed a plasmid library featuring human promoter sequences driving mNeonGreen expression. Promoters were designed using two strategies: the group cochlear promoter A (coProA) : sequences upstream of human HC-specific gene start codons ; and Group cochlear promoter B (coProB): composites of conserved regulatory elements from HC-specific genes and a minimal promoter from human SHH gene. A cross-species screening platform spanning rodents to large animals was established to evaluate these tools.

Results: By using this platform, we identified a set of promoters capable of precisely targeting all HC subtypes: coProA2 (inner HC specific), coProB1 (outer HC specific), and coProB2 (pan HC specific). Notably, coProB2 is the first promoter shown to be effective and safe across three animal models, mice, Bama minipigs, and cynomolgus monkeys,

underscoring its strong translational potential. Using coProB2 to drive expression of Tprn and OTOF, we achieved complete auditory recovery in DFNB79 and DFNB9 mouse models.

Conclusions: We have engineered a cross-species AAV promoter screening platform for precise targeting of cochlear HCs. This system overcomes major delivery barriers in inner ear gene therapy, accelerates clinical translation for sensorineural hearing loss, and establishes a new precision medicine paradigm for genetic deafness.

Comparison of Promising Viral Vectors and Injection Methods for Inner Ear Gene Therapy in a Porcine Large Animal Model

8:00 a.m. - 10:00 a.m.

Erdem Yildiz^{*1}, Katrin Niisuke¹, Till Buschhorn¹, Caroline Sesztak¹, Anselm Joseph Gadenstaetter¹, Matthias Gerlitz¹, Clemens Honeder¹, Hinrich Staecker², Christoph Arnoldner¹, Lukas D. Landegger³

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³*Head and Neck Surgery, Stanford University School of Medicine, Palo Alto, CA, USA*

Background: Gene therapy using adeno-associated viruses (AAVs) has shown promise in treating congenital hearing loss caused by otoferlin mutations. While local gene delivery to the inner ear is feasible, challenges remain regarding delivery-associated trauma, efficacy of different injection methods, and limited in-depth analysis from large animal models. This study set out to analyze AAV-mediated transduction, tropism, and trauma in the porcine cochlea, providing insights into different vector performances in inner ear tissues.

Methods: Pigs received intracochlear injections of AAV2/9-PHP.B-CMV-EGFP via the round window membrane (RWM) with a Hamilton syringe. The collected data from these animals was compared to the results of tropism, transduction efficacy, and hair cell trauma from our previous experiments after AAV2/2-CAG-dTom injection. A novel surgical approach to access the lateral semicircular canal (LSCC) was also established and the AAV2/7M8-CMV-EGFP vector was injected after fenestration of the canal. Different patterns in terms of trauma and transduction were compared between LSCC and RWM injection. Pre- and postoperative brainstem audiometry was performed. After a follow-up of one week, inner ears and other organs relevant for systemic biodistribution were extracted and analyzed by means of immunofluorescence, light-sheet imaging, and confocal microscopy. Additionally, the contralateral ears as well as the peripheral organs were histologically assessed for off-target expression.

Results: A uniform transduction pattern in the immunofluorescent images in all tonotopic regions throughout the cochlea could be observed after PHP.B injection through the RWM with 96.38% of outer hair cells and 97.40% of inner hair cells being transduced. 76.79% of inner and 55.79% outer hair cells were transduced with AAV2 with a basal to apical transduction gradient in the outer hair cells. Mechanical trauma from manual injection was observed but decreased toward the apex. No contralateral or off-target organ transduction could be detected. The LSCC approach was surgically feasible and reproducible; the 7M8 vector also demonstrated promising results based on light-sheet microscopy pending standardized histological validation. Preliminary auditory testing revealed no significant threshold shifts after injection.

Conclusions: The demonstrated efficacy of AAV2 and PHP.B in targeting sensory hair cells supports their reliability as vectors and the pig model provides a preclinical platform to optimize delivery approaches and assess inner ear distribution to refine transduction outcomes. A promising fenestration or injection via the LSCC could potentially offer a reduced trauma after AAV injection. These findings support further development of safe, effective, and clinically relevant strategies for inner ear gene therapy.

Cochlear Injury Reprograms the Cellular Tropism of Synthetic AAV Vectors

8:00 a.m. - 10:00 a.m.

Ayşe Maraslioglu Sperber^{*1}, Rocio Diaz Horstman¹, Tian Wang¹, Lingjun Zhang¹, Alan Cheng¹, Guisheng Zhong², Nesrine Benkafadar¹, Stefan Heller¹

¹Stanford University School of Medicine, ²ShanghaiTech University

Background: Recent breakthroughs in adeno-associated virus (AAV)–based gene therapy have ushered in a new era for treating genetic forms of deafness. These advances emphasize the potential of AAV-mediated strategies for inherited hearing loss and suggest the possibility of using AAV gene delivery to treat acquired forms of deafness caused by ototoxic drugs, noise trauma, or aging. To realize this potential, it is essential to understand how cochlear injury impacts AAV gene delivery. In our study, we used two complementary damage models to investigate how cochlear injury influences AAV transduction and cellular tropism.

Methods: Cochlear injury was induced in one-month-old mice using two paradigms: (i) infusion of a single dose of hyperosmotic sisomicin (ho-sisomicin; 20 g/L; 1.5 µL) into the posterior semicircular canal of FVB mice to achieve synchronous hair cell loss, and (ii) diphtheria toxin (DT; 20 ng/g IP) administration in Pou4f3^{+/DTR} mice to selectively ablate hair cells. One week after damage, AAV-ie-K558R (1.9 × 10¹⁰ genome-containing particles per ear; 1.5 µL) was delivered via posterior semicircular canal injection. In the ho-sisomicin damage model the injection was made through the same opening used previously. Control groups included age-matched FVB and Pou4f3^{+/DTR} mice that received virus without prior damage induction. Cochleae were harvested two weeks post-AAV-injection for immunohistochemical analysis to compare AAV transduction and tropism in damaged versus undamaged tissues.

Results: In undamaged cochleae, AAV-ie-K558R transduced inner hair cells and a subset of spiral ganglion neurons. Following hair cell loss caused by either ho-sisomicin infusion or DT, we observed reporter gene expression in spiral ganglion neurons and, notably, in spiral ligament fibrocytes. The transduction of the spiral ligament exhibited a pronounced basal-to-apical gradient, with higher transduction rates in the basal turn. Importantly, the observation that spiral ligament transduction also occurred in the Pou4f3^{+/DTR} model indicates that this tropism shift is a general result of hair cell loss rather than an artifact of the infusion of a hyperosmolar solution into the inner ear. Contralateral ears served as internal controls. In ho-sisomicin–treated mice, sparse reporter gene expression was observed only in basal-turn inner hair cells, whereas in DT-treated mice, no contralateral transduction was detected.

Conclusions: Our findings show that hair cell loss alters the cochlear microenvironment, causing AAV targeting to shift toward non-sensory cells. This expanded tropism has important implications for gene therapy since the damaged cochlea is the primary target for clinical intervention. Understanding the molecular mechanisms behind this change in tropism

will be key to developing next-generation AAV vectors optimized for treating genetic and acquired hearing loss.

Multimodal Profiling Reveals a Notch-Responsive Regenerative Subpopulation of Cochlear Supporting Cells

8:00 a.m. - 10:00 a.m.

Karen Avraham*¹, Lama Khalaily¹, Shahar Kasirer¹, Katherine Domb¹, Mi Zhou², Shahar Taiber¹, Olga Loza¹, Ran Elkon¹, Litao Tao², David Sprinzak¹

¹Tel Aviv University, ²Creighton University

Background: Regenerative capacity in the mammalian cochlea is extremely limited, and loss of sensory hair cells leads to irreversible hearing deficits. Notch signaling is a key regulator of hair cell differentiation during development, but its role in promoting supporting cell trans-differentiation and the determinants of regenerative competence remain poorly understood. Understanding why only a subset of supporting cells respond to regenerative cues is critical for developing strategies to restore auditory function.

Methods: Neonatal cochlear explants from dual fluorescent reporter mice (Lfng-EGFP;Atoh1-mCherry) were treated with the γ -secretase inhibitor Compound E to inhibit Notch signaling. Live imaging captured dynamic supporting cell behaviors, including morphological remodeling, migration, and spatial pattern disruption. Single-cell multiomic analysis was performed to dissect molecular and epigenetic determinants of supporting cell trans-differentiation at single-cell resolution.

Results: Notch inhibition induced ectopic outer hair cells (OHCs), predominantly derived from Deiters cells (DC), without affecting inner hair cells (IHCs). Live imaging revealed sequential basal displacement, apical migration, and disruption of epithelial patterning, highlighting morphological plasticity beyond fate conversion. The formation of a fourth OHC row reflected both de novo hair cell generation and migration-driven repositioning. Single-cell multiomics identified a transcriptionally and epigenetically distinct “Responsive DC” subpopulation, with activation of hair cell gene programs (Atoh1, Pou4f3, Hes6) and open chromatin at key hair cell loci. In contrast, “Stable DCs” and other supporting cell subtypes remained transcriptionally silent and epigenetically constrained with downregulation of Notch targets. Responsive DCs were enriched for pathways associated with cell adhesion and migration, hair differentiation, reflecting intrinsic competence for fate conversion.

Conclusions: These findings demonstrate that regenerative competence in the neonatal cochlea is confined to a molecularly and morphologically primed supporting cell subpopulation. Notch inhibition alone is insufficient to convert all supporting cells, highlighting intrinsic heterogeneity in regenerative potential. Integration of live imaging with single-cell multiomics provides a mechanistic framework linking signaling inhibition, chromatin accessibility, transcriptional activation, and cellular dynamics. This work identifies fate-competent supporting cells as critical targets for hair cell regeneration and underscores how transcriptional and epigenetic priming, coupled with morphological plasticity, governs regenerative potential in tissues with limited repair capacity.

Symposium 6: Pitch: New Perspectives on a Classic Problem

8:00 a.m. - 10:00 a.m.

Meeting Room 208ABC, Floor 2

Andrew Oxenham, *University of Minnesota*

Symposium Description: Pitch – the perceptual correlate of acoustic repetition rate or fundamental frequency (F0) – is a primary feature of auditory perception, playing a crucial role in speech, music, and animal vocalizations. New experimental results, as well as new methodologies, such as fMRI and machine learning, have solved some long-standing questions, but have opened up new exciting questions for study. This symposium will provide an overview of current state of the art and future directions, by bringing together established and emerging leaders in the field using a wide variety of approaches (human, non-human, behavioral, neural, and modeling). The symposium is targeted at a broad audience with interests from across the ARO, including peripheral and central auditory processing and perception. After opening with an overview of recent findings from human (Oxenham) and non-human (Wang) species, the proposed symposium will continue with an invited talk from an eminent researcher who is not currently a member of the ARO, Professor Isabelle Peretz from the University of Montreal. Dr. Peretz is known for her groundbreaking work on providing a scientific basis for the study of amusia, and for her more recent work on pitch production and improvisation. She will provide an overview, and will be the only speaker to focus on pitch production, rather than just perception. Following these overviews will come presentations of cutting-edge research from junior and mid-level researchers, starting with reports of new fMRI studies of pitch in humans (Tia Ou), findings from intracranial human brain recordings (Joel Berger), non-human behavioral and neurophysiological work (Kerry Walker), and deep neural network modeling approaches to understanding pitch perception and neural coding (Mark Saddler). The symposium will inspire new trans-disciplinary approaches to solving this critical and fascinating problem in auditory research.

Xiaoqin Wang, *Johns Hopkins University*

Human Pitch Perception: Basic Coding and Implications for and of Hearing Loss

Andrew Oxenham, *University of Minnesota*

Neural Representations of Missing Fundamental Harmonic Pitch in the Auditory Cortex of Non-Human Primates

Xiaoqin Wang, *Johns Hopkins University*

Individual Abstract: Experiments in animals have significantly contributed to human studies of pitch perception by revealing how the activity of individual neurons represents harmonic complex and periodic sounds. These studies have shown that the acoustic parameters associated with pitch are encoded by the spiking responses of neurons in various auditory cortical fields. In marmosets, a highly vocal New World primate, a distinct region at the low-frequency border of primary and non-primary auditory cortex contains neurons selective for missing fundamental harmonics. A similar “pitch-center” has been identified in the corresponding location of the human auditory cortex using fMRI. However, a recent fMRI study in macaque monkeys failed to identify such a pitch-center in the auditory cortex. New optical imaging studies suggest that more than one pitch-center may exist in the marmoset auditory cortex. From a computational perspective, it is crucial to distinguish between neurons or cortical regions that extract pitch embedded in harmonic complexes and those that merely carry pitch-related information. This requires examining whether pitch is

specifically or uniquely represented by the neurons or a cortical region under study, as well as verifying that the neural signals correspond to the animal's perception of pitch. In this presentation, I will discuss the progress made since the first identification of a "pitch-center" in marmosets 20 years ago.

Sung Improvisation Reveals Implicit Knowledge of Musical Pitch Across Ages and Abilities

Isabelle Peretz, *University of Montreal*

Individual Abstract: Humans spontaneously invent songs from an early age. Here, we harness this natural inclination to explore implicit pitch knowledge in untrained adult singers and children aged 3 to 8. We analyzed over 8,000 improvised sung melodies, quantifying their pitch content to assess how each reflects tonality—a fundamental organizational principle of music found across cultures. To this end, we developed a new algorithm that offers a high-resolution characterization of melodic structure. The results reflect hallmark features found across global musical traditions reveal, including limited pitch sets, small pitch intervals, and narrow melodic range. Strikingly, tonal signatures emerged even in individuals with congenital amusia, a condition marked by poor performance on explicit musical tasks. These findings demonstrate that sung improvisation is not only engaging but also a powerful tool for uncovering latent musicality across the full range of musical ability and development.

Mapping Cortical Representations of Pitch and Co-Varying Acoustic Features With High-Resolution and Whole-Brain fMRI

Yongtian Ou, *University of Minnesota*

Individual Abstract: Pitch perception arises in complex acoustic environments, where it often covaries with other features such as timbral brightness and temporal dynamics. Functional MRI (fMRI), with its high spatial resolution and broad brain coverage, offers a powerful tool for investigating pitch representations in terms of both fine-grained tuning and spatial distribution across the cortex. We present two fMRI studies that leverage these strengths to probe the cortical encoding of pitch, revealing its relationship with spectral content and spectrotemporal modulation, both within and beyond auditory cortex.

In the first study, we used 3T fMRI to investigate the neural basis of the well-established perceptual confusion between pitch (fundamental frequency, F_0) and timbral brightness (spectral centroid, F_c). We measured BOLD responses when participants listened to harmonic complex tones varying in both F_0 and F_c . Using single-voxel modeling to characterize voxels' tuning properties to F_0 and F_c , we found that the preferred pitch of individual voxels systematically decreased with increasing brightness, and vice versa—consistent with predictions based on perceptual confusion. Furthermore, pitch and brightness mapping share a common high-low-high gradient across auditory cortex, implying a trajectory overlap of cortical activation for changes in each dimension. These findings suggest that pitch and brightness are jointly encoded in a way that reflects natural sound statistics.

The second study used 7T fMRI to explore how pitch and related acoustic features are represented across the whole brain during vowel perception. Participants listened to spoken vowels that varied systematically with respect to vowel identity (probing spectral content), speaker identity (probing F_0 , or absolute pitch), and pitch glide direction (probing spectrotemporal modulation, or relative pitch). Voxel-wise selectivity maps revealed that

auditory cortex was most strongly tuned to vowel identity, with weaker and more diffused selectivity for speaker identity and pitch glide direction. By contrast, premotor cortex and intraparietal sulcus (IPS)—regions beyond auditory cortex— exhibited balanced selectivity across all three features. Multivoxel pattern analysis confirmed that all three features could be decoded from each region, with auditory cortex showing highest accuracy particularly for vowel identity, and non-auditory regions displaying more uniform decoding performance.

Together, these studies demonstrate how fMRI enables precise mapping of pitch representation at both local and global scales. Our findings suggest that pitch encoding in the brain can be shaped by its coexistence with other acoustic features, with auditory cortex exhibiting feature-specific tuning and higher-order regions supporting more integrative or abstract representations. [Supported by NIH grant R01 DC005216.]

What Do Human Intracranial Recordings Tell Us About the Neural Correlates of Pitch Perception?

Joel Berger, *University of Iowa*

Individual Abstract: The perception of pitch is a critical aspect of music, voice perception, and sound segregation, requiring the abstraction of stimulus properties related to the spectrotemporal structure of sound. Previous studies utilizing both animal electrophysiology and human imaging have indicated the presence of a center for pitch representation in the auditory cortex. We have utilized recordings from neurosurgical human patients with epilepsy undergoing intracranial EEG monitoring to identify potentially resectable seizure foci, presenting stimulus sets involving either regular-interval noise (with pitch value determined by repetition rate and pitch strength by temporal regularity) or harmonic complex tones. Recordings of both local field potentials and multi-unit activity (MUA) indicate that pitch-associated responses within the auditory cortex are more widely distributed, covering both posteromedial and anterolateral portions of Heschl's gyrus (Berger et al., 2023; Gander et al., 2019; Griffiths et al., 2010; Kumar et al., 2011). In the most recent study with the highest spatiotemporal resolution, between 62 – 70% of MUA clusters were classified as pitch-responsive based on responses that generalized across different pitch-inducing stimulus conditions. These responses were distributed over Heschl's gyrus. Firing rates increased as a function of the number of iterations of regular-interval noise, consistent with the idea of this being a neural code that represents the perceived salience of the pitch percept. Overall, these findings align with another study performed in a primate model (Kikuchi et al., 2019), as well as others in ferrets (e.g. Bizley et al., 2009, Walker et al., 2011), demonstrating that both local field potential and unit responses to pitch-inducing stimuli appear to be distributed throughout auditory cortex.

Cortical Population Decoding of Pitch Across Harmonic and Temporal Cues

Kerry Walker, *University of Oxford*

Individual Abstract: Pitch is crucial to our experience of musical melody, enhances vocal communication, and helps us focus on one voice in a crowded room. We perceive the pitch of a periodic sound at a single fundamental frequency (F0), and this F0 can be derived from either the regular spacing of harmonic frequencies or the repetition rate of the sound's waveform. While previous studies suggest that auditory cortex may play a key role in processing pitch, it remains unclear how neurons in this region extract F0 from harmonic and temporal features of complex sounds. We performed Neuropixels recordings of single-neuron

spiking activity in the auditory cortex of anaesthetised ferrets while presenting a variety of pitch-evoking sounds that varied in harmonic composition and temporal regularity. We find that F0 is linearly decodable from neural population activity across all sound types presented, but these population codes differ depending on the acoustic content of the sound. Some neurons play a key role in encoding F0 across many different sound types, while other neurons are only informative about a sound's F0 when specific acoustic cues are present. Single neurons with consistent F0 tuning across a variety of pitch cues are often key to population-level F0 encoding across different complex sounds. In these neurons, F0 tuning does not generally correspond to their pure tone tuning. This is recapitulated at the population level, where we find that a linear decoder trained on pure tone responses cannot accurately predict the F0 of complex sounds. Finally, we describe how cortical neurons adapt to two spectrally overlapping streams of complex tones that differ in F0, resulting in oscillations of responses that are dominated by each sound stream. These oscillations are not observed for streams of pure tones presented at the same F0s. Together, our results suggest that subsets of auditory cortical neurons may be specialized to represent the pitch of complex harmonic and temporal pitch cues using mechanisms that cannot be predicted from their pure tone frequency preferences.

Understanding Pitch Perception With Deep Learning

Mark Saddler, *Technical University of Denmark*

Individual Abstract: Advances in machine learning have made it possible to build computational models that perform complex perceptual tasks on par with human listeners. Deep artificial neural networks operating on simulated cochlear input and optimized for ecological hearing tasks have emerged as promising models of human auditory perception. We developed a framework for optimizing such models under different conditions to investigate how perception is constrained by our ears and acoustic environment.

This talk will describe the application of this approach to the domain of pitch perception, whose properties are commonly linked to peripheral neural coding constraints. We first trained artificial neural networks to estimate fundamental frequency (F0) from biologically faithful cochlear representations of natural sounds. Despite never being fit to human data, the networks replicated many characteristics of human pitch judgments. To probe the origins of these characteristics, we then optimized networks with altered cochleae or sound statistics. Human-like behavior emerged only when cochleae had high temporal fidelity and when models were optimized for naturalistic sounds. The results suggest pitch perception is critically shaped by the constraints of natural environments in addition to those of the cochlea.

We next examined the perceptual role of auditory nerve spike timing in speech perception. We optimized models to recognize words and voices, given cochleae with different auditory nerve phase locking limits. Models with access to high-fidelity phase locking produced more human-like behavior than models without, consistent with an essential role in human hearing. Models with phase locking limits below the F0 of most human speech exhibited poorer voice recognition in noise and superhuman robustness to F0 manipulations. These effects suggest that phase locking to the F0 is advantageous for hearing in noise and necessary to explain the dependence of human voice recognition on absolute pitch.

The results illustrate how machine learning models can help clarify the neural coding and environmental factors constraining perception. Our approach has natural extensions to

modeling richer behaviors (such as relative pitch in musical melodies) and the altered perception of individuals with hearing loss and cochlear implants.

Podium 20: Molecular and Cellular Mechanisms Underlying Hair Cell Physiology

10:30 a.m. - 12:30 p.m.

Meeting Room 208ABC, Floor 2

Moderators: Marcos Sotomayor and Jing-Yi Jeng

TMC1 and TMC2 Lipid Scramblase Activity is Regulated by Cholesterol

10:30 a.m. - 10:45 a.m.

Yein Christina Park^{*1}, Hubert Lee¹, Runjia Cui¹, Jayashree Balaraman¹, Angela Ballesteros¹

¹*Section on Sensory Physiology and Biophysics, National Institute on Deafness and other Communication Disorders, Bethesda, MD.*

Background: Mechanoelectrical transduction (MET), the process of converting mechanical stimuli into electric signals, underlies both hearing and balance. The MET machinery resides in the inner ear sensory hair cells and includes transmembrane channel-like proteins 1 and 2 (TMC1 and TMC2), which function as mechanosensitive ion channels. TMC1 mutations affecting MET channel properties lead to hair cell death and subsequent hearing loss, but the mechanism connecting hair cell survival to TMC1 function remains poorly understood. Recent studies suggest that TMC1 and TMC2 may have an additional role as lipid scramblases, facilitating the transbilayer movement of lipids and leading to the externalization of phosphatidylserine (PS), a lipid usually restricted to the inner membrane leaflet. In murine neonatal auditory hair cells, this TMC-dependent lipid scramblase activity is triggered by intracellular calcium chelators and MET channel blockers, while TMC1 deafness-related mutations promote constitutive PS externalization. However, direct evidence for this activity and its regulation have been lacking.

Methods: Here, we demonstrate that purified TMC1 and TMC2 from multiple species exhibit intrinsic lipid scramblase activity when reconstituted into liposomes of controlled lipid composition. Using an in vitro fluorescence-based assay, we show that TMC proteoliposomes containing fluorescently labeled lipids exhibit a greater reduction in fluorescence than empty liposomes upon addition of a membrane-impermeable quencher.

Results: These data indicate that TMC1 and TMC2 facilitate lipid transport between the two liposomal membrane leaflets, consistent with their role as lipid scramblases. Surprisingly, TMC scramblase activity is not directly modulated by calcium nor MET channel blockers, but is tuned by membrane cholesterol. Maximal lipid scramblase activity occurs at 5–10% cholesterol, while both physiological (~30%) or depleted (0%) cholesterol levels hinder TMC scrambling. Notably, several deafness-associated point mutations in TMC1 alter this cholesterol dependence, resulting in hyperactive lipid scrambling at physiological cholesterol levels in vitro and in cochlear explants. Moreover, reducing cholesterol with cyclodextrin in TMC1 mutant cochlear explants significantly decreases the levels of PS externalization.

Conclusions: Together, these findings establish TMC1 and TMC2 as lipid scramblases whose activity is directly regulated by membrane cholesterol. Our work provides a new

mechanistic link between TMC dysfunction and membrane homeostasis, offering insight into the pathogenesis of TMC-related hearing loss.

Myosin Adapter GIPC3 is a Novel Component of the Slow Adaptation Machinery in the Mechanotransducer of Cochlear Outer Hair Cells

10:45 a.m. - 11:00 a.m.

Abigail Dragich*¹, Isabel Aristizabal¹, Savita Sharma¹, Mary Freeman¹, Shadan Hadi¹, Craig Vander Kooi², Gregory Frolenkov¹

¹University of Kentucky, ²University of Florida, College of Medicine

Background: Decrease of mechano-electrical transduction (MET) current during step deflection of the hair bundle is known as adaptation, which can be separated into “slow” and “fast” components. Slow adaptation seems to be driven by stereocilia myosin(s), because it is affected by non-selective inhibitors of myosin’s ATPase cycle. Furthermore, selective inhibition of MYO1C disrupts slow adaptation in mouse vestibular but not auditory hair cells; the latter may use MYO7A instead. One of the top MYO7A-associated proteins identified in mouse stereocilia is GIPC3, a member of Gα-Interacting Protein, C-terminus (GIPC) family of proteins that can couple myosin motors with their membrane or cellular cargoes. GIPC3 mutations cause non-syndromic hearing loss in humans. Thus, the goal of this study was to explore the role of GIPC3 in cochlear hair cell stereocilia.

Methods: We generated two new mouse strains: i) without GIPC3 (*Gipc3*^{-/-}); and ii) with a truncation mutation (*Gipc3*^{W301X}) recapitulating a human mutation causing severe deafness. Auditory brainstem responses (ABRs) assessed hearing thresholds, while scanning electron microscopy examined hair bundle morphology and tip links. Whole-cell patch clamping was used to investigate MET currents evoked by fluid-jet or rigid-probe deflections of hair bundles in P4-P8 cochlear hair cells. High-resolution (< 1.5nm/pixel) focused-ion beam scanning electron microscopy (FIB-SEM) examined stereocilia ultrastructure in lower (LTLD) and upper (UTLD) tip link density regions, the proposed locations of slow adaptation machinery. New GIPC3 antibodies were generated to localize GIPC3 together with other stereocilia proteins by immunofluorescence. In parallel, biochemical and structural biology techniques were used to assess GIPC3 structure and in vitro interactions.

Results: Homozygous mice of both *Gipc3* mutant strains developed profound hearing loss by P35-40 and, by P7, exhibited apparently identical stereocilia bundle abnormalities with preserved tip links in both inner (IHCs) and outer (OHCs) hair cells. GIPC3 was found at 1st row stereocilia and near the tips of transducing 2nd row stereocilia in wildtype and *Gipc3*^{W301X/W301X} hair cells but disappeared in *Gipc3*^{-/-} mice. OHCs lacking one copy of full-length *Gipc3* (*Gipc3*^{+/-} or *Gipc3*^{W301X/-}) had decreased extent of slow adaptation that worsened in *Gipc3*^{-/-} mice. The extent of slow adaptation did not change in *Gipc3*^{W301X/W301X} OHCs, consistent with our in vitro assays indicating that this truncation eliminates autoinhibition but does not disrupt PDZ-binding domain of GIPC3. These assays also showed GIPC3 interaction with C-terminus of CDH23. Consistently, we observed the loss of UTLDs and mis-localization of MYO7A from OHC bundles of *Gipc3*^{-/-} but not *Gipc3*^{W301X/W301X} mice. In contrast to OHCs, *Gipc3*^{-/-} IHCs exhibited no changes in the extent of slow adaptation, had normal UTLDs, and correct localization of MYO7A.

Conclusions: GIPC3 is a novel component of slow adaptation machinery in OHCs that is likely to reside in the UTLD and includes MYO7A.

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In Silico Hierarchical and Calcium-Dependent Mechanics of Inner-Ear Tip Links Under Resting Tension

11:00 a.m. - 11:15 a.m.

Haosheng Wen^{*1}, Carter T. Wheat², Jasanvir S. Sandhu², Elakkiya Tamilselvan², Pedro De-la-Torre², Joseph C. Sudar³, Travis Harrison-Rawn², Marissa Boyer², Qurat Ashraf¹, Marcos Sotomayor¹

¹*The University of Chicago*, ²*The Ohio State University*, ³*New York University Medical Center*

Background: Tip links, formed by cadherin-23 (CDH23) and protocadherin-15 (PCDH15), convey mechanical forces to pull open inner-ear mechanotransduction channels. To date, no direct in silico, in vitro, or in situ measurement of the elasticity of the full tip-link ectodomain has been reported. Thus, whether tip links act as soft or stiff gating springs remains controversial.

Methods: We constructed two complete atomic models of the tip-link ectodomain using X-ray crystallographic structures and AlphaFold predictions that include the CDH23 membrane-adjacent domain MAD28 in monomeric or dimeric form. These models were used for all-atom molecular dynamics (MD) simulations systematically investigating the calcium-dependent dynamics and mechanics of tip links in the absence and the presence of resting tension.

Results: Equilibrium simulations of our tip link models fully loaded with calcium showed CDH23 and PCDH15 conformations consistent with previously reported electron microscopy images. We monitored overall twisting, localized hotspots of flexibility, and fluctuations at non-canonical extracellular cadherin (EC) linkers (CDH23 EC12-13 and EC27-MAD28; PCDH15 EC3-4, EC5-6, EC9-10, and EC11-MAD12). A tip link system with reduced calcium showed high flexibility at all linker regions.

Constant-velocity stretching simulations starting from equilibrium conformations (absence of resting tension) predicted a two-phased mechanical response with soft (~1 mN/m) and stiff components (up to 30 mN/m) at 0.1 nm/ns in the presence of calcium. The soft response was attributed to extension of flexible linkers and straightening of PCDH15 EC9-10. The tip-link mechanical response with reduced calcium occupancy still had two phases but both were significantly softer.

Constant-force simulations mimicking resting tension at 20 and 40 pN showed that both models of tip links extend over 10 nm, with effective spring constants varying from 1.2 to 2.8 mN/m. A novel ladder-like dimerization involving CDH23 EC25–EC27 and MAD28 was also observed. Constant-velocity simulations of pre-stretched tip links (presence of high 40 pN resting tension) at 0.1 nm/ns showed only a stiff elastic response.

Analyses of stretching trajectories revealed a reproducible hierarchy of mechanical events across systems: (i) straightening and extensions of various flexible linkers and the PCDH15 EC9-10 kink, (ii) unrolling and unfolding of CDH23 MAD28s, and (iii) subsequent unrolling and unfolding of PCDH15 MAD12s.

Conclusions: We present the first set of all-atom simulations of the entire tip link ectodomain. Our data show that the mechanical response of the heterotetrameric CDH23 and PCDH15 complex is non-linear and highly dependent on calcium and resting tension, which

suggests that the tip-link remains as a good candidate to be a soft gating spring in inner-ear mechanotransduction.

In Vivo Genetic Manipulation of SNAP-25 Uncovers Its Critical Role in Exocytosis at Inner Hair Cell Ribbon Synapses

11:15 a.m. - 11:30 a.m.

Maxence Cornille^{*1}, Najate Benamer¹, Mauricio Saenz-Roldan¹, Marie-José Lecomte¹, Didier Dulon¹, Saaid Safieddine¹

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Background: The inner hair cell (IHC) ribbon synapse is specialized for the rapid and sustained release of neurotransmitters required for high-fidelity auditory signaling. We have shown that SNAP-25, a core component of the neuronal SNARE complex, is essential for this process, highlighting its critical role in mediating IHC exocytosis (Calvet et al., 2022). Ex vivo studies in chromaffin cells have elucidated key mechanisms of SNAP-25 function, where specific mutations such as the C-terminal truncation SNAP-25 Δ 9 and the Q174L point mutation severely impair vesicle fusion (Wei et al., 2000). Although these studies established the molecular importance of SNAP-25 in neuroendocrine cells, it remains unclear whether such disruptive effects extend to the highly specialized ribbon synapse of IHCs in vivo. Investigating hearing outcomes following virally delivered SNAP-25 variants directly in IHCs offers a powerful approach to link molecular perturbations of the SNARE machinery with auditory function assessed in the intact, genetically modified animal model.

Methods: Viral constructs and delivery: GFP-tagged SNAP-25 variants (wild-type, Δ 9 truncation, Q174L substitution) were packaged into AAV8 vectors. Neonatal C57BL/6 mice (P2) received cochlear injections via the round window.

Immunohistochemistry: The cochleae were fixed and organs of Corti were immunolabeled for GFP, Myosin7A, and CTBP2/Ribeye. Confocal imaging was used for localization and ribbon synapse counts.

Electrophysiology: Whole-cell patch-clamp recordings from GFP-positive IHCs assessed calcium currents and exocytosis by capacitance measurements. Calcium uncaging was performed to probe vesicle pool dynamics.

Auditory testing: ABRs and DPOAEs were recorded to assess auditory thresholds and OHC function.

Results: All exogenous GFP-SNAP25 variants localized predominantly to the IHC membrane. Overexpression of GFP-SNAP25 did not affect calcium currents, exocytosis, or hearing thresholds, confirming that the GFP tag does not interfere with SNAP-25 function. In contrast, expression of SNAP-25 Δ 9, induced profound hearing loss despite preserved outer hair cell function. This phenotype was associated with an almost complete loss of exocytosis in IHCs. Expression of SNAP-25Q174L caused moderate hearing loss with selective impairment of the sustained-release pool while sparing the readily releasable pool. Quantification of ribbon synapses revealed that both SNAP-25 Δ 9 and SNAP-25Q174L reduced synapse numbers, with the strongest effect observed for SNAP-25 Δ 9. Together, these results show that SNAP-25 variants differentially disrupt IHC synaptic exocytosis in vivo through distinct mechanisms that mirror ex vivo findings, leading to hearing loss of varying severity and directly linking SNARE complex dysfunction to auditory deficits.

Conclusions: Our findings demonstrate that SNAP-25 is essential for IHC synaptic exocytosis and hearing. While GFP-SNAP25 overexpression was functionally neutral, SNAP-25 Δ 9 caused profound deafness by abolishing IHC exocytosis, and SNAP-25Q174L produced moderate deficits by selectively impairing sustained release. These in vivo results corroborate ex vivo chromaffin cell and in vitro data, establishing a direct link between SNAP-25 dysfunction, ribbon synapse integrity, and auditory impairment.

Visualizing How Presynaptic Activity Shapes Ribbon Synapse Formation in Zebrafish Hair Cells

11:30 a.m. - 11:45 a.m.

Olivia Molano*¹, Katie Kindt¹

¹*National Institute on Deafness and Other Communication Disorders, National Institutes of Health*

Background: Ribbon synapses are essential for the rapid and precise transmission of sensory information from hair cells. At ribbon synapses, the voltage-gated calcium channel Cav1.3 plays a critical role in mediating neurotransmission. While the function of Cav1.3 at mature ribbon synapses is relatively well characterized, its role in synapse formation remains less clear. Research in zebrafish has shown that during development, spontaneous Cav1.3-dependent calcium events influence ribbon synapse formation (Wong et. al., 2019, Sheets et al., 2012). Disruption of these calcium events results in an excessive number of small presynaptic ribbons, enlarged and abnormal postsynaptic structures, and ultimately, a loss of synaptic integrity. More recent work in zebrafish has revealed the dynamics of ribbon formation (Hussain et. al., 2024). Here small ribbon precursors migrate and fuse on microtubules to reach the active zone. We hypothesize that Cav1.3-dependent activity during development is required to regulate the dynamic processes of ribbon formation and to stability maintain complete ribbon synapses.

Methods: We are studying ribbon and synapse formation in vivo, in larval zebrafish when larvae are 2 days old and the majority of hair cells and synapses are forming. To visualize hair cells, their microtubule networks, ribbons and precursors, as well as postsynaptic structures, we use transgenic lines expressing florescent markers. Using these transgenic lines, in combination with Zeiss Airyscan confocal microscopy, we can capture ribbon precursor movement and fusion events, and synapse assembly over the course of 1-2 hours.

Results: For our initial analyses, we focused on ribbon formation, specifically examining both ribbon migration along microtubules and fusion events. We found when Cav1.3 is blocked, presynaptic ribbons can migrate along microtubules to reach the active zone but fail to stability fuse. Ribbon precursors make repeated attempts to fuse with each other, but fusions are transient and ultimately unsuccessful. These findings suggest that Cav1.3-mediated activity is critical for regulating the fusion of ribbon precursors during development. To extend this analysis, we are now using live imaging to capture the dynamics of how ribbons and postsynaptic structures assemble at the active zone. Preliminary results indicate that Cav1.3 mutants develop abnormal postsynaptic structures early in the hair cell's lifespan and form fewer complete synapses.

Conclusions: Understanding how ribbon synapses form is important because synaptic elements can detach from the active zone and change in size and location after damage. Thus, by uncovering how Cav1.3-dependent activity regulated the assembly and stabilization of ribbon synapses, our work provides insights into the developmental mechanisms that

establish functional sensory circuits. These insights many ultimately inform strategies to repair or reform ribbon synapses after damage, with the goal of preserving or restoring sensory system function.

Calcium Imaging as a Window Into Vestibular Inner Ear Physiology

11:45 a.m. - 12:00 p.m.

Marina Kabirova*¹, Christopher Luong¹, Olivia Lutz¹, Dana Silvian¹, Kehan (Doris) Zhang¹, Anvy Kuriakose², Aravind Chenrayan Govindaraju², Robert M. Raphael², Ruth Anne Eatock¹

¹*University of Chicago*, ²*Rice University*

Background: The utricle, a vestibular end organ, detects linear acceleration and head tilt and has two zones: central (striola) and peripheral (extrastriola). Striolar afferents emphasize precise spike timing for high-frequency stimuli, while extrastriolar afferents rely more on firing rate for encoding low-frequency motion. Each afferent integrates signals from populations of hair cells (HCs) and the populations differ between zones in, e.g., their receptive field dimensions and relative contributions of different HC types. Despite these well-documented differences, little is known about how populations of hair cells and afferents respond moment-by-moment to stimuli. Addressing this gap is critical for understanding the nature of vestibular signals to the brain.

Methods: We used ex vivo preparations of the mouse utricle (P2-P35), with GCaMP8m expressed selectively in HCs or afferents. Mechanotransduction was activated in HC populations by fluid-jet stimuli at frequencies from 0.1-20 Hz. Some imaging fields included striolar and extrastriolar regions, allowing simultaneous comparison across zones. Single-cell patch-clamp recordings were combined with calcium imaging to directly link receptor potentials to fluorescence changes. To enable cross-preparation comparisons and build a map of the sensory epithelium, we developed an approach to accurately assign each recorded region of interest (ROI) to its location and to register ROIs into a canonical utricle framework.

Results: Single-cell patch recordings provided insight into the relationship between voltage change and calcium signals. Preliminary analyses suggest that Cav1.3 channels positioned near ribbon synapses in the basal HC are the primary sites of voltage-dependent calcium entry. Calcium entry through MET channels can be visualized also and is well-known to be physiologically relevant, but changing whole-cell voltage did not detectable change resting levels of calcium in hair bundles.

We identified a previously undescribed form of intercellular coupling: when a single HC is depolarized with whole-cell voltage clamp, neighboring unstimulated cells can also show (smaller) calcium responses. This coupling suggests a local communication mechanism, possibly through the extracellular medium.

Population calcium imaging revealed that both HCs and afferents were more responsive to high-frequency stimulation (> 2 Hz) in striola than in extrastriola, consistent with previous information from single-afferent recordings. We were also able to capture fluid-jet evoked calcium flow in hair bundles.

Conclusions: Through single-cell patching plus calcium imaging, we provide information needed to deconvolve HC and afferent calcium signals into voltage signals. This approach also has uncovered unexpected intercellular coupling between neighboring HCs. Population

data highlight zonal differences in tuning. The ability to map ROIs on to a canonical utricular epithelium enables us to build up a map of the epithelium with data from multiple preparations. This work deepens our understanding of how vestibular signals are represented on the vestibular epithelium.

Sound Encoding Properties of Inner Hair Cells in the Living Mouse Cochlea

12:00 p.m. - 12:15 p.m.

Mark Presker*¹, Siyang Lin¹, Gerardo Molina¹, Kevin Ohlemiller¹, Edward Han¹, Jinkyung Kim¹

¹*Washington University School of Medicine*

Background: The cochlea is an exceptionally sensitive and precise sensory organ, capable of detecting sounds ranging from a ticking clock to a blasting siren. Inner hair cells (IHCs), the primary sensory cells for hearing, convert sound vibrations into electrical signals, transmitting auditory information to the brain. How IHCs encode the vast range of sound intensities, spanning more than six orders of magnitude, remains one of the most remarkable yet elusive features of the auditory system. While direct in vivo monitoring of the peripheral cellular dynamics has advanced our understanding of visual, olfactory, gustatory, and tactile sensory encoding, such high-resolution intravital interrogation of the auditory system has proven extremely challenging due to the cochlear complex, fragile, and fluid-filled structure, while being deeply embedded in the temporal bone. Owing to these challenges, a knowledge gap in sound encoding exists between the mechanical vibrations at the organ level and the cellular activity at the single cell/nerve level. To bridge this gap, we developed a hearing-preserved in vivo two-photon cochlear imaging method that enables direct characterization of the receptive properties of individual IHCs while retaining normal hearing function.

Methods: We monitor sound-evoked Ca²⁺ responses of individual IHCs using two-photon cochlear imaging in Myo15Cre-driven GCaMP8s mice while simultaneously presenting sounds. The number of activated IHCs, their activity levels ($\Delta F/F_0$), and response patterns are quantified across varying sound frequencies and intensities. We also record auditory brainstem response (ABR) pre- and post-surgery, and at the conclusion of the experiments, to ensure stable hearing sensitivity.

Results: Individual IHCs exhibit frequency tuning curves shaped around their characteristic frequency (CF), the frequency at which they are most responsive. As sound intensity increases, more IHCs are recruited, each showing progressively larger Ca²⁺ responses. At high intensities, individual IHCs respond to a broader range of frequencies; however, their response amplitudes decrease as the stimulus frequency moves further from their CF along the tonotopic axis. The dynamic range of IHC Ca²⁺ responses is maximal at the CF and gradually diminishes with increasing tonotopic distance. Similarly, activity among neighboring IHCs is strongly correlated near the CF, with correlation strength declining exponentially over distance. Direct cell-to-cell comparisons reveal that clusters of 3–5 contiguous IHCs exhibit highly similar response patterns.

Conclusions: Our in vivo data reveal how individual IHCs contribute to encoding sound frequency and intensity, providing new insights for understanding peripheral auditory processing. A deeper understanding of the receptive property will also advance strategies to replicate the ear's remarkable sensitivity and selectivity in engineered devices, which

currently face limitations in spectral resolution, speech perception in noise, and music appreciation, ultimately benefiting over 430 million people with disabling hearing loss.

ATP2a3 Maintains Inner Hair Cell Function by Controlling Endoplasmic Reticulum Calcium Recycling

12:15 p.m. - 12:30 p.m.

Yiyun Lou¹, Jiaoyao Ma¹, Mingyu Xia², Wenyan Li^{*1}

¹The EENT Hospital of Fudan University, ²Eye and ENT Hospital, Fudan University

Background: Intracellular calcium ion (Ca^{2+}) homeostasis plays a critical role in maintaining cellular function and viability, with endoplasmic reticulum (ER)-resident calcium channels and pumps being of particular importance. While the contribution of Ca^{2+} channels in hair cells (HCs) to auditory processes—such as mechanoelectrical transduction (MET) adaptation and synaptic vesicle release—is well established, the specific role of ER-mediated calcium regulation in auditory function remains poorly understood. In this study, we identified the ER calcium ATPase gene ATP2a3 as specifically expressed in inner hair cells (IHCs); however, its function in Ca^{2+} homeostasis and IHC physiology had not previously been characterized.

Methods: Through analysis of snRNA-seq data from the adult mouse cochlea, we delineated the expression profile of ATP2a3. To further characterize its spatiotemporal expression dynamics within the auditory system, we performed RNAscope in situ hybridization and utilized an ATP2a3-HA knock-in mouse model. An Atp2a3-deficient mouse model was generated using CRISPR-Cas9 technology to evaluate its impact on auditory function, cochlear morphology, and cellular physiology. Transmission electron microscopy (TEM) and whole-cell patch-clamp recordings were employed to examine the subcellular architecture and electrophysiological properties of inner hair cells (IHCs), including ribbon synapses, ER structures, and functional characteristics in different age groups. To determine whether Atp2a3 deficiency increases noise susceptibility, we evaluated relevant phenotypes using a noise-induced hearing loss model. Real-time monitoring of intracellular and ER Ca^{2+} dynamics was performed using AAV-mediated GCaMP6s and D1ER expression under noise exposure and Atp2a3 deficiency. Furthermore, we overexpressed Atp2a3 in cochlear organoids via lentivirus and employed Co-IP-MS to identify potential interaction partners of SERCA3, the protein encoded by Atp2a3.

Results: ATP2a3/SERCA3 expression initiates in basal turn inner hair cells at E18.5, stabilizes postnatally by P1, and persists into adulthood with IHC-specificity throughout the auditory pathway. Progressive high-frequency hearing loss arises from three months of age and worsens with aging. Atp2a3-deficient mice display heightened susceptibility to moderate-intensity noise exposure. TEM revealed that ATP2a3 deficiency resulted in structural disruptions of ribbon synapses and fragmentation of the endoplasmic reticulum in IHCs. Electrophysiological recordings showed reduced Ca^{2+} currents, along with impaired sustained synaptic vesicle release and recycling in Atp2a3-deficient IHCs. Ca^{2+} imaging further demonstrated a significant decrease in both the frequency and amplitude of Ca^{2+} activity in these cells. Additionally, proteomic interaction analysis identified several potential binding partners of SERCA3.

Conclusions: This study elucidates the specific expression and functional role of endoplasmic reticulum calcium regulator ATP2a3/SERCA3 in IHCs. Targeting ATP2a3 represents a potential therapeutic strategy for the treatment of hearing loss and establishes a

molecular basis for precision medicine interventions aimed at modulating calcium homeostasis in IHCs.

Special Session 4: Hearing Science at the Technological Frontier: Tools, Insights, and Integration

10:30 a.m. - 12:30 p.m.

Ballroom B, Floor 3

Hearing Science at the Technological Frontier: Tools, Insights, and Integration

Seba Ausili, *University of Miami*

All Together Now: A Live Demo of a Hearing Research Pipeline

Tobias Goehring¹, Mareike Buhl², Jessica Monaghan³, Seba Ausili⁴, Fotios Drakopoulos⁵

¹*University of Zurich*, ²*Hearing Institute, Institut Pasteur, Paris, France*, ³*National Acoustic Laboratories*, ⁴*University of Miami*, ⁵*University College London*

Tobias Goehring, *University of Zurich*

Individual Abstract: This closing segment will demonstrate how the tools presented throughout the session can be combined into a fully integrated experimental pipeline for hearing research. By connecting methods for online testing, data standardization, large language models (LLMs), modern laboratory infrastructure, and deep neural networks (DNNs), this demonstration will illustrate how innovation across domains can converge into a powerful, modular framework.

We begin with a remote auditory task, where a simulated participant completes an online test, such as a speech-in-noise related task. The resulting behavioral data is structured using standardized formats to ensure compatibility and reusability across systems. This standardized data is then passed to a large language model (LLM). The LLM generates a natural-language summary of the participant's performance, offers a preliminary interpretation of their auditory profile, and proposes a follow-up testing strategy tailored to the results.

This recommendation is then sent to a lab designed for multisensory and ecologically valid experiments. Stimuli are adapted in real time based on the LLM output and presented using a dynamic setup that supports head tracking, spatialized sound, and immersive configurations. The lab segment demonstrates how hardware and software components can be coordinated to support adaptive experimentation.

Finally, the data collected from the lab is processed using a deep neural network (DNN) trained to model auditory behavior. The model predicts performance patterns, classifies listener profiles, and provides individualized insights based on observed responses.

Together, these steps form a complete chain, demonstrating how emerging technologies can be applied not only in isolation but in combination. This integrative demo will provide

attendees with a clear and practical vision of how to implement modern tools into real-world auditory research workflows.

Conversing With Data: Using Large Language Models to Streamline Auditory Research

Jessica Monaghan¹

¹*National Acoustic Laboratories*

Jessica Monaghan, *National Acoustic Laboratories*

Individual Abstract: Large language models (LLMs) are opening new possibilities for automating and scaling interpretation tasks in auditory science and clinical audiology. This talk explores their role within an integrated research pipeline, where LLMs receive structured data from remote hearing tasks, such as audiograms and speech-in-noise scores, and generate both human-facing summaries and machine-readable outputs to guide subsequent testing.

In the research context, I will demonstrate how an LLM can synthesize a participant's behavioural and audiometric data into a clear, natural-language performance summary, paired with a structured recommendation for follow-up. These outputs serve two roles: one as a report for researchers or clinicians, and another as a directive for automated lab systems to adapt stimuli, spatial setups, or sequencing in real time. The LLM thus acts as a reasoning agent, making context-sensitive decisions that enable modular and participant-specific experimentation.

This approach is also highly applicable to clinical settings. The ability to generate draft reports, interpret performance trends, and recommend next tests has direct relevance for clinical audiology, where time and expertise are often constrained. Here it is even more important that automation can be accomplished safely and transparently. I will present strategies for ensuring that LLM outputs clearly indicate areas requiring human oversight, particularly for safety-critical aspects like stimulus levels, and highlight how uncertainty or ambiguity can be flagged for review.

This focus on research automation and clinical potential illustrates how LLMs can support both exploratory experimentation and decision support in hearing care. By producing transparent, structured, and context-aware outputs, we can use LLM as intelligent agents to connect data, reasoning, and action. The talk will include examples from live pipelines, demonstrating how we can use these technologies safely, effectively, and ethically to enhance both research and clinical practice.

Building Smart Labs: Flexible Setups for Spatial and Real-Time Auditory Experiments

Seba Ausili¹

¹*University of Miami*

Seba Ausili, *University of Miami*

Individual Abstract: As we prepare for the next wave of technological change, auditory researchers need experimental spaces that evolve just as fast. The goal is a “plug-and-play” vision: a core lab framework you can use today yet expand tomorrow with the latest sensors, renderers, or AI modules—without tearing everything apart.

This talk answers four practical questions:

1 - What are the essential building blocks of a modern auditory lab? We will map out the signal chain—from low-latency audio interfaces and loudspeaker arrays to motion-tracking cameras and eye trackers—showing how each component affects timing accuracy and ecological validity.

2 - What alternatives exist at different price points? Side-by-side comparisons will cover off-the-shelf micro-controllers, networked DSP boxes, and high-end multi-speaker amps, with clear guidance on when a USD 1 k solution suffices and when a USD 50 k upgrade is justified.

3 - Which hardware choices give the biggest performance gains? Data from case studies will be presented to illustrate key trade-offs in system design, including latency, recording precision, and participant comfort. These real-world examples will highlight how different hardware and synchronization choices affect experimental performance and data quality.

4 - How do we keep everything synchronized? We will discuss how a unified software layer, built on open-source synchronization protocols, can align audio, video, motion, and physiological data with sub-millisecond precision, regardless of the specific hardware or programming environment in use.

The emphasis is on enabling high-level science while staying within realistic budgets. I will outline cost-conscious strategies for selecting and repurposing hardware, discuss how open-source software and community-shared designs can lower barriers to entry, and highlight the importance of publishing schematics, code, and benchmarks so that other labs can replicate, refine, and build upon the work.

Beyond the Booth: Remote Testing and the Rise of Personalized Hearing Science

Tobias Goehring¹, Robert Carlyon²

¹*University of Zurich*, ²*Cambridge Hearing Group, University of Cambridge*

Tobias Goehring, *University of Zurich*

Individual Abstract: Web-based applications are increasingly used in clinical audiology, driven by the development of mobile, remote technology and strong demand. Remote applications have large potential to increase statistical power, accessibility and diversity in research studies and to support audiology services. We aimed to bring these advantages to hearing sciences and cochlear implant (CI) research and developed a web-based listening test system called AUDITO. By exploiting wireless streaming technology and personal mobile devices, AUDITO can be used to flexibly implement and administer a wide range of listening tests remotely or in-the-lab. The system was designed to be easy to use without software programming apart from optional stimuli pre-generation. Experimenters can exchange listening test setups and stimuli sets within the system to facilitate collaboration across labs, institutions and countries. Technical features were implemented to ensure signal quality over wireless streaming. The AUDITO system facilitates data collection, enables research collaboration and can improve accessibility and inclusion in CI research to enable patient-centred care and personalized hearing science. The lessons learned from the development of the system and first AUDITO studies will be discussed together with the benefits and challenges of remote testing more in general.

Bridging Audiology and Data Science: Toward Standardized and Scalable Hearing Profiles

Mareike Buhl¹, Samira Saak², Marta Campi¹, Eugen Kludt³, Lena Schell-Majoor², Antje Wulff⁴, Birger Kollmeier², Paul Avan¹

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Mareike Buhl, *Institut Pasteur, Université Paris Cité, Inserm, Institut de l'Audition, IHU reConnect, F-75012, Paris, France*

Individual Abstract: A large variety of data is collected in clinical as well as research settings, including studies with large numbers of patients. These data are typically collected in local databases, exhibiting different data structure, choice of tests, or varying data quality. If these data were analyzed together, knowledge about many patients worldwide could be exploited to obtain a representative overview of existing patient patterns, and classifications could provide likelihoods for hearing loss or most appropriate hearing device categories to support clinical decision-making.

Our auditory profiling approach aims at characterizing patients in audiological databases by combinations of audiological test results in an unsupervised approach, such as audiogram, speech test, and loudness scaling (Saak et al., 2022). The resulting distinct auditory profiles describe the groups included in the employed database, hence, the profiles cannot be generalized. As a way out, Saak et al. (2025, accepted) developed an approach for merging the generated profiles obtained from several databases to capture all included profiles. The combined profiles extend the range of test outcomes without sharing individual data. Hence, by applying this approach to large patient numbers from different databases, a set of generalized – and eventually standardized - auditory profiles could be achieved.

However, such a scaling to many data sources crucially depends on the available format and content of the data. Interoperability therefore plays a key role which assures common understanding and meaning of the data described by data models and terminologies, allowing for machine-actionable evaluation of whether the database content is compatible.

The EFAS working group “Data formats for Big Data in Audiology” is developing audiological data models (archetypes) based on the openEHR data standardization approach, for example, for audiogram, speech test, and loudness scaling. The archetypes contain all data and metadata that are required to describe the collected data, such as stimuli, measurement equipment, or target variables of a test. For a speech test, this can be the SRT as outcome of adaptive measurements or several datapoints describing a psychometric function.

In summary, the combination of auditory profiling and standardized audiological data leverages the potential of achieving hearing loss profiles that characterize population-representative accounts of clinical (and research) data. In a practical application, individual data collected in standardized format, if in the lab, in clinics or online, could be classified into profiles, and additional tests or provision with appropriate hearing devices could be recommended depending on the respective individual profile.

Learning to Listen: General-Purpose Deep Networks for Human Hearing

Fotios Drakopoulos¹

¹*University College London*

Fotios Drakopoulos, *University College London*

Individual Abstract: Developing models that replicate human auditory behaviour has been a long-standing goal in hearing science. Such models can serve as a valid *in silico* surrogate for listeners and can be used to develop personalised hearing technologies that are tailored to the hearing profile of an individual.

This talk outlines how general-purpose models for human auditory perception can be developed by answering three main questions:

1. How can deep learning be used to model human auditory behaviour? We will discuss the shortcomings of the state of the art and the challenges that need to be addressed to yield deep learning systems that achieve natural (rather than objectively optimal) performance.
2. How can existing knowledge be used to develop general-purpose models? We will discuss how foundation models of auditory processing can be used to derive generic models of auditory behaviour that are personalised with only a few trainable parameters. The goal is not only to develop accurate models of auditory behaviour, but also models that can be easily personalised to reflect the hearing idiosyncrasies of individuals and to accurately simulate their performance patterns.
3. What datasets need to be collected to build such models? While large-scale datasets might be necessary for the general-purpose models, small datasets will be sufficient to individualise these models. Successfully predicting individualised outcomes depends on the careful design of the listening tests that will form these datasets, with the aim of capturing all the necessary information for quantifying the auditory profiles of individual listeners, with and without hearing pathologies.

General-purpose models of hearing have the potential to advance current sound technologies and to yield new tools for hearing science. But this can only be done by advancing the existing clinical setups and by building synergies between different labs, bringing together expertise across audiology, neuroscience, hearing technology and psychoacoustics.

Podium 21: Development: Auditory and Vestibular Circuit Assembly

10:30 a.m. - 12:30 p.m.

Meeting Room 209ABC, Floor 2

Moderators: Jaime Garcia-Anoveros and Esther Fousert

Early and Late Forming Vestibular Ganglion Neurons Are Positionally and Transcriptionally Distinct

10:30 a.m. - 10:45 a.m.

Zachary Stoner^{*1}, Doris K. Wu²

¹National Institute on Deafness and other Communication Disorders, ²Section on Sensory Cell Regeneration and Development, Laboratory of Molecular Biology, National Institute on Deafness and Other Communication Disorders, National Institutes of Health

Background: The vestibular ganglion (VG) is a key component in mediating the sense of balance and is comprised of bipolar afferent vestibular ganglion neurons (VGNs). VGNs innervate the five vestibular end organs of the inner ear and the brainstem/cerebellum centrally. VGNs as well as their spiral ganglion counterparts originate from the otocyst where highly ordered gene regulatory networks orchestrate their production. Neurog1 positive, neural-fated cells delaminate from the otic epithelium, adopt either a vestibular or auditory fate, and eventually form two anatomically distinct ganglia. Much work has been done to tease apart the principles behind auditory neuron identity and diversification. However, the mechanisms regulating VG organization and identity are not clear. Prior studies in chick and zebrafish suggest that there is a temporal sequence to the respective vestibular and statoacoustic ganglion organization. The organization of the VG in mammals, however, is not known and does not appear to be segregated according to the targeted sensory organs. To address mechanisms underlying VGN organization and fate determination, we investigated whether the order in which VGNs are generated regulates their positions within the VG and their transcriptional identities.

Methods: We utilized Neurog1CreERT2 transgenic mice to lineage-trace neuronal-fated cells. 4-Hydroxytamoxifen (4-OHT) was injected at a single timepoint between E8.5 to E11.5 to pregnant RosatdTomato female mice crossed to Neurog1CreERT2 males. Then, embryos were harvested at E16.5 and analyzed for their position within the VG. Additionally, the transcriptional profiles of these temporally distinct VGN populations were conducted using scRNA-seq on Neurog1CreERT2; RosatdTomato VG collected at E16.5. The sequenced results were mapped to a house mouse reference genome with the WPRE sequence added to detect tdTomato expression. Furthermore, scRNA-seq was also conducted on CVG/VG collected at E9.5, E10.5, E11.5, E13.5 and postnatal day P111 from Neurod1Cre;RosatdTomato embryos/adults. Analyses were performed using Seurat and Slingshot.

Results: Lineage tracing and scRNA-seq results at E16.5 using Neurog1CreERT2 demonstrated that

VGN organization and transcriptional identities are temporally distinct. Specifically, VGNs produced early were localized at dorsal and ventral poles of the VG and as subsequent neurons were produced, they were progressively added towards the center. Early- and late-forming VGNs showed different transcriptional profiles. Using trajectory inference, we mapped signature genes and tdTomato expression of these early and late VGNs to scRNA-seq results from earlier ages. We found that these early and late VGNs can be traced to distinct lineages which begin to branch at E9.5, and these lineages remained segregated in the adult VG.

Conclusions: Our results indicate that early and late-forming VGNs occupy different spatial positions within the VG, and they exhibit different transcriptional profiles. Furthermore, our results suggest that neuronal identities in the VG could be pre-determined by E9.5, well ahead of when neuroblast delamination is complete.

Inner and Outer Hair Cell Effects on Cochlear Circuit Formation

10:45 a.m. - 11:00 a.m.

Charles Murphey*¹, Berta Soria-Izquierdo², Junhua Tan¹, Alexandra L. Woo¹, Colin O. Brame¹, Ignacio García-Gómez¹, Annie B. Nguyen¹, Jianghong Wang¹, Yingjie Zhou¹, Mary Ann Cheatham¹, Jaime García-Añoveros¹

¹*Northwestern University*, ²*Northwestern University and The Cajal Institute of the Consejo Superior de Investigaciones Científicas*

Background: The cochlea contains two types of mechanosensors, inner (IHC) and outer (OHC) hair cells, which perform distinct functions (transmission and amplification) and are differentially innervated: IHCs synapse onto spiral ganglion type I afferents, which are in turn axodendritically innervated by lateral olivocochlear (LOC) efferents, while OHCs are innervated by spiral ganglion type II afferents and medial olivocochlear (MOC) efferents. We wondered what the roles of IHCs and OHCs may be in the assembly of this auditory neural circuit. The transcription factor TBX2 is a master regulator of inner vs outer hair cell identity: elimination of TBX2 from IHCs converts them into OHCs of the inner compartment (ic-OHCs), whereas ectopic TBX2 converts OHCs into IHCs of the outer compartment (oc-IHCs) (García-Añoveros et al. 2022, Nature). We previously explored the effects on innervation of OHC to oc-IHC conversions in *Insm1* knockouts, and we inferred a sequential hierarchy of commands from hair cells to afferents and from afferents to efferents in circuit formation (Webber et al. 2021, Science Advances). Here, we further test and expand on this model by exploring the effects of the conversions caused by TBX2 removal or addition.

Methods: We used *Tbx2* conditional knockout (cKO) mice with a variety of Cre lines (*Gfi1*-Cre, *Atoh1*-Cre, and tamoxifen-inducible *Fgf8*-CreER and *Vglut3*-CreER) to ablate floxed *Tbx2* and convert IHCs to ic-OHCs at different developmental stages. We also used *Gfi1*-Cre; *R26*-LSL-*Tbx2*-mCherry mice to induce ectopic TBX2 overexpression embryonically and convert nearly all OHCs into oc-IHCs. In all models, we then used immunohistochemical markers to examine type I and II cochlear afferent innervation and synapse formation.

Results: IHC to ic-OHC conversion with *Tbx2* cKOs embryonically resulted in retraction of growing type I afferents, which remained in contact with inner border and phalangeal cells but not with ic-OHCs. Postnatal conversions up to P9 resulted in type I afferents maintaining contact with ic-OHCs but having no synapses. With later conversions (P11), type I afferents maintained contact and synapses with ic-OHCs. Type II afferents appear to be unchanged in these *Tbx2* cKOs, innervating OHCs in the outer compartment but not ic-OHCs. Embryonic OHC to oc-IHC conversion with ectopic TBX2 resulted in some type I cochlear afferents traversing the tunnel of Corti into the outer compartment and contacting oc-IHCs.

Conclusions: Type I afferents transiently contact ic-OHCs but are repelled during a critical perinatal period. Further, ic-OHCs prevent formation of stable synapses during the critical period of P9-P11, but do not eliminate them once formed. Our results also confirm that embryonic IHCs can be innervated by type I afferents even if in the outer compartment. Finally, ic-OHCs are not sufficient for attracting type II afferent innervation, as outer supporting cells might be also needed.

Spatial Mapping of Single-Cell Sequencing Data Reveals Lineage-Specific Compartments in the Mouse Otocyst

11:00 a.m. - 11:15 a.m.

Xinyu Bao¹, Saikat Chakraborty¹, Brett Mehling¹, Jie Liu¹, Joerg Waldhaus*¹

¹*University of Michigan*

Background: The otocyst, the embryonic precursor to the inner ear, gives rise to nearly all epithelial cell types that line its fluid-filled chambers, including the specialized sensory epithelia of the organ of Corti, the cristae, and the otolith-bearing maculae. While previous studies have characterized gene expression across broad regions of the mouse otocyst at embryonic day 10.5 (E10.5), the spatial and molecular identity of progenitor populations committed to individual sensory organs at the otocyst stage remains poorly defined.

Methods: To address this, we generated a high-resolution single-cell RNA sequencing (scRNA-seq) dataset comprising 1,556 otocyst cells at E10.5, with an average sequencing depth of 17,010 reads per cell.

Results: Using a modified version of an established computational approach, we reconstructed a three-dimensional transcriptional map of the otocyst, enabling spatial localization of cell populations based on gene expression signatures. Hierarchical clustering identified five distinct cellular clusters, which were mapped to specific anatomical regions within the 3D otocyst model. Spatial accuracy was validated both in silico, through visualization of canonical marker gene expression, and by comparison with experimental otocyst staining. To connect early progenitor domains to later inner ear structures, we integrated our dataset with five previously published scRNA-seq datasets covering subsequent developmental stages and offering organ-level resolution. Applying trajectory inference and metadata-guided digital lineage tracing, we mapped the emergence of organ-specific progenitors to defined regions on the otocyst surface. Notably, we identified a small, spatially restricted population of putative organ of Corti progenitors near the ventral pole of the otocyst. Further analysis revealed asymmetric gene expression within the cochlear compartment, suggesting that the compartmental boundaries at the otocyst stage play an instructive role in the establishment of graded gene expression along the prospective apex-to-base axis.

Conclusions: In summary, by integrating high-depth single-cell transcriptomics with spatial and temporal reconstruction, we reveal the early lineage segregation of organ-specific progenitors in the otocyst with high resolution. These findings offer valuable insights to study inner ear development and inform strategies for generating organ-specific tissues in vitro using organoid-based or stem cell-derived systems.

Visualizing the Contribution of α -Neurexin3 in Ribbon Synapse Assembly

11:15 a.m. - 11:30 a.m.

Yommi Tadesse*¹, Lucia Salatino¹, Katherine Pinter¹, Katie Kindt¹

¹*National Institute of Health, NIDCD*

Background: To transmit sensory signals, hair cells form specialized ribbon synapses with afferent neurons. While loss of ribbon synapses leads to hearing and balance impairments, the molecular mechanisms underlying ribbon synapse assembly remain poorly understood. Previous work in our lab has identified α -Neurexin3 (α -Nrxn3) as a necessary molecule in ribbon synapse assembly in both mice and zebrafish; however, how α -Nrxn3 alters the dynamics of ribbon synapse assembly remains unclear.

Methods: We utilize the zebrafish lateral line (LL) system to study hair cells and ribbon synapse assembly. The LL sensory system is easily accessible for in vivo imaging, and LL synapses mature rapidly (12-18 hrs). To investigate the dynamics of LL synapse formation in relation to α -Nrxn3, we generated transgenic lines labeling hair cells, pre-, and post-synapses in both α -Nrxn3 and wild-type animals. We are using these lines to acquire in vivo, high-

resolution Airyscan images at various stages of ribbon synapse development in both genotypes. For our imaging we have acquired timelapses at two intervals (every 4 min for 2 hrs and every 30 min for 5-6 hrs) to track ribbon synapse development over time.

Results: In wild-type animals, synaptic components pair to form, on average, three synapses per LL hair cell. Timelapse analysis reveals that at the earliest stage wild-type hair cells form paired synapses primarily via pairing of small, pre- and post-synaptic components. Interestingly, at later stages, we observe more complex fission and fusion events that results in an increase or decrease in paired synapses. In contrast, we found that developing pre- and post-synaptic components in α -Nrnx3 mutants fail to stably pair. This results in an excess of unpaired presynapses throughout development, with only one paired synapse per hair cell forming on average. In addition, over time, in α -Nrnx3 mutants, unpaired postsynapses retreat and accumulate in the terminal ends after hair cell maturation.

Conclusions: We demonstrate several processes that define ribbon synapse formation in vivo. In addition, we show that α -Nrnx3 is required to stably assemble ribbon synapses. Identifying molecules necessary for ribbon synapse formation and understanding the mechanisms by which they function is essential for developing novel therapeutic targets to treat hidden hearing loss caused by disrupted ribbon synapse assembly.

Semaphorin-5A Regulates Spiral Ganglion Neuron Activity in the Developing Cochlea

11:30 a.m. - 11:45 a.m.

Satish Ghimire^{*1}, Nidhee Seernaum¹, Reza Amanipour², Trinh Nguyen³, Rachel Chelstein¹, Alex Flynn¹, Emily Gibbons¹, Ronna Hertzano², Dwight Bergles³, Thomas Coate¹

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Background: The development of sensory networks depends on genetic programming, and both spontaneous and stimulus-driven neural activity. Spontaneous activity in the pre-hearing cochlea causes spiral ganglion neurons (SGNs) to fire bursts of action potentials, which are necessary for auditory circuit formation. However, the mechanisms that regulate spontaneous firing in the cochlea are not well understood. In this study, we determined that SEMA5A, a membrane-tethered semaphorin expressed by SGNs, controls their excitability to modulate their structural and functional maturation in the developing cochlea.

Methods: Sema5A expression in the developing cochlea was determined using in situ hybridization and immunohistochemistry. To determine SEMA5A function, we performed Ca²⁺ imaging using genetically encoded Ca²⁺ sensors in postnatal day 2 (P2) cochlear explants from Snap25-GCaMP6s+ transgenic mice. SEMA5A-Fc was acutely applied to modulate SEMA5A function, and activity in Sema5a knockout (KO) and wildtype (WT) cochleae were also compared. SGN survival, morphology, hair cell synapse formation and type I SGN subtype density were also examined to determine the consequences of Sema5a loss. Variants of recombinant SEMA5A were applied to determine if specific amino acid residues (predicted to interact with ion channels) mediate the effects of SEMA5A on SGN activity. Finally, to examine functional consequences of loss of Sema5a, auditory brainstem response (ABR) and juxtacellular recordings from SGNs in excised cochleae were performed.

Results: Immunostaining revealed that SEMA5A mainly localizes to SGN cell bodies. Acute activation of SEMA5A through application of SEMA5A-Fc to cultured cochleae eliminated

SGN Ca²⁺ transients, and Sema5a KO cochleae showed increased and sustained Ca²⁺ fluorescence in SGNs (compared to WT), suggesting SEMA5A normally reduces SGN activity. Consistent with this hypothesis, juxtacellular recordings revealed that Sema5a KO SGNs exhibited higher rates of burst firing and more spikes per burst compared to controls. Spontaneous supporting cell activity in the cochlear epithelium was unchanged by these manipulations, indicating SEMA5A acts directly on SGNs. Since SEMA5A shares partial sequence homology with hanatoxin, a known inhibitor of potassium and Ca²⁺ channels, it is possible that SEMA5A interacts with ion channels in this process. Indeed, applying a mutant SEMA5A-Fc with the hanatoxin-like domain deleted, did not alter SGN activity.

Analysis of SGN morphology revealed that these changes in activity in Sema5a KO mice were associated with defects in dendritic refinement. In addition, Sema5a KO mice showed overall reduced SGN density and CALB2 positive SGNs at P28. However, ABR recordings showed that Sema5a KO mice had normal auditory thresholds across tested frequencies, indicating no significant defects in hearing acuity.

Conclusions: Our data suggest SEMA5A regulates spontaneous neural activity in the cochlea during development, which is critical for controlling SGN dendritic refinement, survival and differentiation. Our findings also suggest that SEMA5A may influence SGN excitability potentially by interacting with ion channels.

Role of Mechanotransduction and Phospholipid Flippase ATP8A2 in Outer Hair Cell Afferent Synapse Pruning

11:45 a.m. - 12:00 p.m.

Katherine Nimchuk^{*1}, Aisha Hasan¹, David Lee¹, Jung-Bum Shin²

¹*University of Virginia*, ²*University of Virginia School of Medicine*

Background: For hearing to occur it is necessary that the sensory hair cells (HCs) of the cochlea form functional connections with afferent spiral ganglion neurons (SGNs). In early development there are excessive neurons and synapses within the cochlea that must be removed prior to the onset of hearing in a process called pruning. Presently very little is known about how pruning happens. We hypothesize that phosphatidylserine (PS), a common “eat me” signal, marks the neurons and synapses to be removed during pruning. In homeostatic conditions, PS is relegated to the inner leaflet of the cell membrane by flippases. Flippases are proteins that drive cell membrane asymmetry by shuttling lipids like PS from the outer leaflet of the cell membrane to the inner leaflet. There are several flippases known to be expressed in the cochlea, indicating that one or more of them could be necessary for synaptic pruning by preventing essential neurons and synapses from dying. Flippase ATP8A2 is known to be necessary for hearing function, and we therefore hypothesize that ATP8A2 may be necessary for pruning. We observed a marked shift in ATP8A2 localization within the cochlea during the pruning period, with a distinct concentration at outer hair cell (OHC) synapses and type II SGNs.

Methods: We investigated the role of ATP8A2 in the developing cochlea by generating several mouse lines. Atp8a2-HA mice have an HA tag knocked-into the C-terminus of ATP8A2 for visualization of the protein. We knocked out Cib2 to generate a mouse line with hair cells that do not perform MET (Cib2^{-/-}) and crossed this line to our Atp8a2-HA line to visualize the localization of ATP8A2 in MET negative mice. To directly test the necessity of the flipping function of ATP8A2 in pruning, we generated a mouse with hypomorphic alleles of ATP8A2 (A2Δ33 mice). We performed immunostaining for CtBP2 and quantified the

number of synapses/HC with confocal microscopy to determine how these proteins impact pruning.

Results: *Cib2*^{-/-} mice showed a significant change in the localization of ATP8A2 and OHC pruning was disrupted in these mice compared to WT. Synapse numbers in A2Δ33 mice were not significantly different from WT mice at hearing onset, but by P21 A2Δ33 mice had fewer OHC synapses than WT mice. At 1 month old the A2Δ33 mice exhibited elevated auditory brainstem response thresholds.

Conclusions: These data indicate that OHC pruning is dependent on mechanotransduction and similarly that MET impacts the localization of ATP8A2. ATP8A2 is necessary for the survival of OHC synapses and A2Δ33 mice had hearing loss at one month old compared to controls. Future work will test for potential compensation of partial loss of function of ATP8A2 by other cochlear flippases.

Characterizing the Excitatory to Inhibitory Shift in GABA Regulation of Mouse Neonatal Cochlear Development

12:00 p.m. - 12:15 p.m.

Holly Beaulac^{*1}, Sian Kitcher¹, Catherine Weisz²

¹National Institute on Deafness and Communication Disorders, ²National Institutes of Health

Background: Cochlear innervation by afferent and efferent pathways forms the canonical central-peripheral auditory circuitry. The spiral ganglion contains type I and type II afferent neurons (SGNs) within the cochlea while efferent medial olivocochlear (MOC) neurons of the central auditory system project directly to the cochlea. MOC efferents contact the outer hair cells and form cholinergic synapses. MOC efferents additionally release GABA, which in the developing cochlea can activate presynaptic GABAB receptors and downregulate cholinergic activity. We have previously shown that GABA release from MOC efferents can also act on GABAA receptors in type II SGNs pre- and post-hearing onset. It is established that intraneuronal chloride concentration is high in the neonatal brain; when ionotropic GABA or glycine channels open, negatively charged chloride ions exit, depolarizing the cell. This excitation via GABA receptors is thought to have roles in synapse and neuronal development. As the brain matures, the upregulation of specific chloride transporters extrudes chloride, allowing GABA receptor activation to hyperpolarize the cell, resulting in inhibition. During neonatal sensory development, does GABA produce similar early excitatory activity in cochlear neurons that later transitions to inhibitory activity? During cellular excitation, intracellular calcium increases via various processes. Therefore, we used calcium imaging as a proxy for cellular excitation to characterize the spatiotemporal changes in GABA activity in the developing mouse cochlea.

Methods: To examine calcium responses in SGNs following exogenous GABA application, we utilized two strategies: (1) at postnatal day (P)2, we injected C57BL/6J pups in the posterior semicircular canal with AAV-GCaMP7f, a fast calcium indicator, and (2) we bred transgenic *Bhlhb5Cre*; *SalsaGCaMP6f* mice, to express the calcium indicator in SGNs. Using confocal microscopy, we imaged ex vivo cochleae at P4-15 to evaluate changes in calcium activity in response to 10-30 seconds of 1mM GABA application.

Results: The change in fluorescence in SGN dendrites was quantified for 1:30-3 min (2.6-10 fps) with intensity relative to baseline conditions of tissue bathed in [5.8 mM K⁺] solution. GABA induced increases in AAV-GCaMP7f fluorescence in P4, P7, and P10 cochleae (n=1-3). Small to no changes were seen at P11 and P15, respectively (n=1-3). GABA-induced

increases in fluorescence were visible in the transgenic GCaMP6f mice at P5-8, but not at P9 or older (n=1-7).

Conclusions: Preliminary results suggest that GABA excites type II SGNs early in development. A decline in excitation is observed before P11, suggesting that, like other neurons, chloride concentrations decline in SGNs during development. In the mature auditory system, the action of GABA on cochlear neurons is likely inhibitory.

Formation of Tonotopic Connections in the Auditory Brainstem

12:15 p.m. - 12:30 p.m.

Daniel Pederick^{*1}, Yunming Wu², Stephen Quake², Liquan Luo²

¹*Johns Hopkins School of Medicine*, ²*Stanford University*

Background: Auditory perception is critical for communication through speech and music. The pitch or

frequency of a sound is processed by the brain using tonotopic maps, which are the spatial organization of how sounds with different physical frequencies are represented in all auditory regions. Tonotopic connections are formed during development and further refined by sound-evoked activity after the onset of hearing. It is unclear what molecules and mechanisms guide the assembly of tonotopic maps before the onset of hearing and how these are disrupted in neurodevelopmental disorders.

Methods: Using Smart-seq2 we profiled excitatory neurons from embryonic day 17 cochlear nucleus and

inhibitory neurons from embryonic day 18 superior olivary complex using Slc17a6-Cre;Ai14 and En1-

Cre;Ai14 mice to gain genetic access, respectively.

Results: Recently, we identified that the cell surface molecules Teneurin-3 (Ten3) and Latrophilin-2 (Lphn2)

display tonotopic expression gradients in multiple regions of the developing auditory brainstem. Ten3 is expressed in low frequency responding regions and Lphn2 is expressed in high frequency responding regions. We leveraged Ten3 and Lphn2 expression levels to assign cells to distinct positions across the tonotopic axis and identified genes that displayed specific tonotopic expression patterns. We identified multiple molecules that show tonotopic expression patterns in the cochlear nucleus, superior olivary complex or both. This included cell surface molecules, transcription factors and RNA-binding molecules with a subset being associated with neurodevelopmental disorders. This dataset provides a resource to understand how these connections are assembled by cell surface molecules and how those cell surface molecules are regulated by transcription factors.

Conclusions: Here, we present the first molecular map of tonotopy in the developing auditory brainstem. We

have identified a number of molecules with novel tonotopic expression patterns. This will provide the

foundation for future studies investigating how tonotopy is established during development and how these connections are disrupted in neurodevelopmental disorders.



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